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<b>(54) Title:</b> SEED PLANTS CHARACTERIZED BY DELAYED SEED DISPERSAL  <b>(57) Abstract</b>  The present invention provides a non-naturally occurring seed plant that is characterized by delayed seed dispersal due to ectopic expression of a nucleic acid molecule encoding an AGL8-like gene product. Further provided herein is a non-naturally occurring seed plant, such as an <i>ag11 ag15</i> double mutant, that is characterized by delayed seed dispersal due to suppression of AGL1 and AGL5 expression in the seed plant. The invention also provides a substantially purified dehiscence zone-selective regulatory element, which includes a nucleotide sequence that confers selective expression upon an operatively linked nucleic acid molecule in the valve margin or dehiscence zone of a seed plant. Also provided by the invention are kits for producing a transgenic seed plant characterized by delayed seed dispersal, such kits containing a dehiscence zone-selective regulatory element.		

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**SEED PLANTS CHARACTERIZED BY DELAYED SEED DISPERSAL**

This invention was made with government support under DCB9018749 awarded by the National Science Foundation. The government has certain rights in the  
5 invention.

**BACKGROUND OF THE INVENTION****FIELD OF THE INVENTION**

The present invention relates generally to plant molecular biology and genetic engineering and more  
10 specifically to the production of genetically modified seed plants in which the natural process of dehiscence is delayed.

**BACKGROUND INFORMATION**

Rapeseed is one of the most important oilseed  
15 crops after soybeans and cottonseed, representing 10% of the world oilseed production in 1990. Rapeseed contains 40% oil, which is pressed from the seed, leaving a high-protein seed meal of value for animal feed and nitrogen fertilizer. Rapeseed oil, also known as canola  
20 oil, is a valuable product, representing the fourth most commonly traded vegetable oil in the world.

The production of oilseeds, meal and oil from rapeseed plants has been increasing continuously for the last 30 years for food and feed grains, mainly by

expansion of the area under cultivation. Most northern European countries produce rapeseed as their main edible oil crop. By the year 2000, China is expected to be the leading producer with 9.2 metric tons (Mt; 26%); followed  
5 by India with 7.8 Mt (22%); the European Community (12 countries), with 7.6 Mt (21%); Canada, 3.8 Mt (11%) and eastern Europe with 2.6 Mt (7%).

Unfortunately, the yield of seed from rapeseed and related plants is limited by pod dehiscence, which is  
10 a process that occurs late in fruit development whereby the pod is opened and the enclosed seeds released. Degradation and separation of cell walls along a discrete layer of cells dividing the two halves of the pod, termed the "dehiscence zone," result in separation of the two  
15 halves of the pod and release of the contained seeds. Seed "shattering," whereby seeds are prematurely shed through dehiscence before the crop can be harvested, is a significant problem faced by commercial seed producers and represents a loss of income to the industry. Adverse  
20 weather conditions can exacerbate the process of dehiscence, resulting in greater than 50% loss of seed yield.

Attempts to solve this problem over the past 20 years have focused on the breeding of shatter-resistant  
25 varieties. However, these plant hybrids are frequently sterile and lose favorable characteristics that must be regained by backcrossing, which is both time-consuming and laborious. Other strategies to alleviate pod shattering include the use of chemicals such as pod  
30 sealants or mechanical techniques such as swathing to reduce wind-stimulated shattering. To date, however, a simple method for producing genetically modified seed



plants that do not open and release their seeds prematurely has not been described.

Thus, a need exists for identifying genes that regulate the dehiscence process and for developing  
5 genetically modified seed plant varieties in which the natural seed dispersal process is delayed. The present invention satisfies this need and provides related advantages as well.

#### SUMMARY OF THE INVENTION

10 The present invention provides a non-naturally occurring seed plant that is characterized by delayed seed dispersal due to ectopic expression of a nucleic acid molecule encoding an AGL8-like gene product. The AGL8-like gene product can have, for example,  
15 substantially the amino acid sequence of an AGL8 ortholog such as *Arabidopsis* AGL8 (SEQ ID NO:2). Particularly useful seed plants of the invention, which are characterized by delayed seed dispersal, include members of the *Brassicaceae*, such as rapeseed, and members of the  
20 *Fabaceae*, such as soybeans, peas, lentils and beans.

In one embodiment, the invention provides a transgenic seed plant that is characterized by delayed seed dispersal due to ectopic expression of a nucleic acid molecule encoding an AGL8-like gene product. In a  
25 transgenic seed plant of the invention, the nucleic acid molecule encoding the AGL8-like gene product can be operatively linked to an exogenous regulatory element. Useful exogenous regulatory elements include constitutive regulatory elements and dehiscence zone-selective  
30 regulatory elements. In particular, the exogenous regulatory element can be a dehiscence zone-selective

regulatory element that is an *AGL1* regulatory element or an *AGL5* regulatory element.

In another embodiment, the invention provides a non-naturally occurring seed plant that is characterized  
5 by delayed seed dispersal due to suppression of both *AGL1* and *AGL5* expression in the seed plant. Such a non-naturally occurring seed plant characterized by delayed seed dispersal can be, for example, an *agl1 agl5* double mutant.

10 The present invention further provides a tissue derived from a non-naturally occurring seed plant of the invention. In one embodiment, the invention provides a tissue derived from a non-naturally occurring seed plant that has an ectopically expressed nucleic acid molecule  
15 encoding an *AGL8*-like gene product and is characterized by delayed seed dispersal. In another embodiment, the invention provides a tissue derived from a non-naturally occurring seed plant in which *AGL1* expression and *AGL5* expression each are suppressed, where the seed plant is  
20 characterized by delayed seed dispersal.

Methods of producing a non-naturally occurring seed plant characterized by delayed seed dispersal also are provided herein. Such methods entail ectopically  
expressing a nucleic acid molecule encoding an *AGL8*-like  
25 gene product in the seed plant, whereby seed dispersal is delayed due to ectopic expression of the nucleic acid molecule.

The invention also provides a substantially purified dehiscence zone-selective regulatory element,  
30 comprising a nucleotide sequence that confers selective expression upon an operatively linked nucleic acid

molecule in the valve margin or dehiscence zone of a seed plant, provided that the dehiscence zone-selective regulatory element does not have a nucleotide sequence consisting of nucleotides 1889 to 2703 of SEQ ID NO:4.

- 5 The dehiscence zone-selective regulatory element can be, for example, an *AGL1* regulatory element or *AGL5* regulatory element.

Further provided is a plant expression vector containing a dehiscence zone-selective regulatory element  
10 that confers selective expression upon an operatively linked nucleic acid molecule in the valve margin or dehiscence zone of a seed plant, provided that the dehiscence zone-selective regulatory element does not have a nucleotide sequence consisting of nucleotides 1889  
15 to 2703 of SEQ ID NO:4. If desired, a plant expression vector can contain a nucleic acid molecule encoding an *AGL8*-like gene product in addition to the dehiscence zone-selective regulatory element.

The invention also provides a kit for producing  
20 a transgenic seed plant characterized by delayed seed dispersal, such kit containing a dehiscence zone-selective regulatory element that confers selective expression upon an operatively linked nucleic acid molecule in the valve margin or dehiscence zone of a seed  
25 plant, provided that said dehiscence zone-selective regulatory element does not have a nucleotide sequence consisting of nucleotides 1889 to 2703 of SEQ ID NO:4. In a kit of the invention, the dehiscence zone-selective regulatory element can be, if desired, operatively linked  
30 to a nucleic acid molecule encoding an *AGL8*-like gene product.

### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a scanning electron micrograph of an *Arabidopsis* gynoecium at about the time of pollination. A number of distinct cell types are shown, including the apical stigma, the style, and the ovary. The ovary walls, or valves, which are separated along their entire lengths by a small suture denoted the "replum," are indicated. The dehiscence zone, a narrow band of cells one to three cells wide along the valve/replum boundary, also is indicated.

Figure 2 shows a wild type *Arabidopsis* fruit immediately following pod shattering. The seeds as well as the replum are clearly visible.

Figure 3 shows scanning electron micrographs of wild type *Arabidopsis* and a representative 35S::AGL8 transgenic line. The dehiscence zone is evident in the wild type plant. In contrast, in the 35S::AGL8 transgenic line, the cells of the outer replum are converted to a valve cell fate, and the dehiscence zone is absent.

Figure 4 shows the *agl5* and *agl1* genomic regions and the loss of *AGL5* or *AGL1* expression, respectively, in the *agl5* or *agl1* mutant. Figure 4A shows the genomic structure of the *AGL5* gene, with the positions of exons indicated by boxes, and the positions of introns indicated by thin lines. The *agl5* mutant allele, generated by targeted disruption following homologous recombination, has a kanamycin resistance cassette that is indicated by a yellow hatched box and located within the MADS-box region. Figure 4B shows the

genomic structure of the *AGL1* gene, with the position of the approximately 17 kb T-DNA insertion into the large intron of the *agl1-1* locus indicated by the arrowhead. Exons are indicated by boxes. Introns are indicated by thin lines. The MADS-box region is shown as a hatched box. Figure 4C shows that a probe specific for the 3' end of the *AGL5* complementary cDNA detected the *AGL5* transcript in wild type but not in the *agl5* knockout mutant plants. Figure 4D shows that a probe specific for the 3' end of the *AGL1* complementary DNA (cDNA) detected the *AGL1* transcript in wild type but not in the *agl1* mutant generated by T-DNA insertion.

Figure 5 shows scanning electron micrographs of wild type *Arabidopsis* and an *agl1 agl5* double mutant. The valves are beginning to detach from the replum in the wild type *Arabidopsis* fruits, which are shown during the process of dehiscence. At the same time in development, the valves of the *agl1 agl5* double mutant plant remain attached to the replum.

Figure 6 shows the nucleotide (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequence of *Arabidopsis* AGL8.

Figure 7 shows the nucleotide sequence of the *Arabidopsis* *AGL1* gene (SEQ ID NO:3). The exons and translation start site are indicated.

Figure 8 shows the nucleotide sequence of the *Arabidopsis* *AGL5* gene (SEQ ID NO:4). The exons and translation start site are indicated.

### DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a non-naturally occurring seed plant that is characterized by delayed seed dispersal due to ectopic expression of a nucleic acid molecule encoding an AGL8-like gene product. The AGL8-like gene product can have, for example, substantially the amino acid sequence of an AGL8 ortholog such as *Arabidopsis* AGL8 (SEQ ID NO:2).

The fruit, a complex structure unique to flowering plants, mediates the maturation and dispersal of seeds. In most flowering plants, the fruit consists of the pericarp, which is derived from the ovary wall, and the seeds, which develop from fertilized ovules. *Arabidopsis*, which is typical of the more than 3000 species of the *Brassicaceae*, produces fruit in which the two carpel valves (ovary walls) are joined to the replum, a visible suture that divides the two carpels. The structure of an *Arabidopsis* gynoecium around the time of pollination, including the carpel valves and replum, is shown in Figure 1.

Pod dehiscence or shatter occurs late in fruit development in a wide spectrum of important plant crops such as oilseed rape (*Brassica napus* L.) and is a process of economic importance that can lead to significant losses in seed yield. In oilseed rape, dehiscence involves the breakdown of cell wall material in a discrete cell layer known as the "dehiscence zone," which is a region of only one to three cells in width that extends along the entire length of the valve/replum boundary (Meakin and Roberts, *J. Exp. Botany* 41:995-1002 (1990)). As the cells in the dehiscence zone separate



from one another, the valves detach from the replum, allowing seeds to be dispersed (see Figure 2).

The plant hormone ethylene is produced by developing seeds and appears to be an important regulator of the dehiscence process. One line of evidence supporting a role for ethylene in regulation of dehiscence comes from studies of fruit ripening, which, like fruit dehiscence, is a process involving the breakdown of cell wall material. In fruit ripening, ethylene acts in part by activating cell wall degrading enzymes such as polygalacturonase (Theologis et al., Develop. Genetics 14:282-295 (1993)). Moreover, in genetically modified tomato plants in which the ethylene response is blocked, such as transgenic tomato plants expressing antisense polygalacturonase, there is a significant delay in fruit ripening (Lanahan et al., The Plant Cell 6:521-530 (1994); Smith et al., Nature 334:724-726 (1988)).

In dehiscence, ultrastructural changes that culminate in degradation of the middle lamella of dehiscence zone cell walls weaken rapeseed pods and eventually lead to pod shatter. As in fruit ripening, hydrolytic enzymes including polygalacturonases play a role in this programmed breakdown. For example, in oilseed rape, a specific endo-polygalacturonase, RDPG1, is upregulated and expressed exclusively in the dehiscence zone late in pod development (Petersen et al., Plant Mol. Biol. 31:517-527 (1996), which is incorporated herein by reference). Ethylene may regulate the activity of hydrolytic enzymes involved in the process of dehiscence as it does in fruit ripening (Meakin and Roberts, J. Exp. Botany 41:1003-1011 (1990), which is incorporated herein by reference). Yet, until now, the

proteins that control the process of dehiscence, such as those regulating the relevant hydrolytic enzymes, have eluded identification.

The present invention is directed to the surprising discovery that the AGL8 transcription factor regulates the process of dehiscence. As disclosed herein, *Arabidopsis* plants were transformed with an AGL8 cDNA under control of a 35S cauliflower mosaic virus (CaMV) constitutive promoter such that AGL8 was ectopically expressed throughout the transformed plant. In particular, AGL8, which is normally expressed in the carpel valves, was ectopically expressed in the replum, which is a small strip of cells separating the two valves in a mature fruit. As a consequence of such ectopic expression, the replum of the fruit was absent, with the cells of the outer replum replaced by cells having characteristics of valve identity, demonstrating that, in this context, AGL8 expression is sufficient to specify valve cell fate. Furthermore, ectopic expression of the AGL8 cDNA produced a transgenic plant in which the dehiscence zone failed to develop normally, resulting in delayed seed dispersal (see Example I). Whereas wild type *Arabidopsis* produced fruit that opened and released seeds on or about 14 days after pollination, transformed *Arabidopsis* ectopically expressing AGL8 produced fruit in which seed dispersal was postponed, or in which the seeds were never released unless the fruit was opened manually (see Figure 3). Thus, for the first time, seed plants were genetically modified to delay the natural process of dehiscence.

The present invention also relates to the surprising discovery that an *agl1 agl5* double mutant seed plant has a delayed seed dispersal phenotype that is

strikingly similar to the AGL8 gain-of-function phenotype. As disclosed herein, loss-of-function mutations in the *AGL1* and *AGL5* genes were produced by disruptive T-DNA insertion and homologous recombination (see Example II). In the resulting *agl1 agl5* double mutant plants, the dehiscence zone failed to develop normally, and the mature fruits did not undergo dehiscence (see Figure 5). Thus, *AGL1* or *AGL5* gene expression is required for development of the dehiscence zone. These results indicate that *AGL1*, *AGL5* and *AGL8* regulate pod dehiscence and that manipulation of *AGL1*, *AGL5* and *AGL8* expression can allow the process of pod shatter to be controlled.

Thus, the present invention provides a non-naturally occurring seed plant that is characterized by delayed seed dispersal due to ectopic expression of a nucleic acid molecule encoding an AGL8-like gene product. The AGL8-like gene product can have, for example, substantially the amino acid sequence of an AGL8 ortholog such *Arabidopsis* AGL8 (SEQ ID NO:2).

As used herein, the term "non-naturally occurring," when used in reference to a seed plant, means a seed plant that has been genetically modified by man. A transgenic seed plant of the invention, for example, is a non-naturally occurring seed plant that contains an exogenous nucleic acid molecule encoding an AGL8-like gene product and, therefore, has been genetically modified by man. In addition, a seed plant that contains, for example, a mutation in an endogenous AGL8-like gene product regulatory element or coding sequence as a result of calculated exposure to a mutagenic agent, such as a chemical mutagen, or an "insertional mutagen," such as a transposon, also is

considered a non-naturally occurring seed plant, since it has been genetically modified by man. In contrast, a seed plant containing only spontaneous or naturally occurring mutations is not a "non-naturally occurring seed plant" as defined herein and, therefore, is not encompassed within the invention. One skilled in the art understands that, while a non-naturally occurring seed plant typically has a nucleotide sequence that is altered as compared to a naturally occurring seed plant, a non-naturally occurring seed plant also can be genetically modified by man without altering its nucleotide sequence, for example, by modifying its methylation pattern.

The term "ectopically," as used herein in reference to expression of a nucleic acid molecule encoding an AGL8-like gene product, refers to an expression pattern that is distinct from the expression pattern in a wild type seed plant. Thus, one skilled in the art understands that ectopic expression of a nucleic acid encoding an AGL8-like gene product can refer to expression in a cell type other than a cell type in which the nucleic acid molecule normally is expressed, or at a time other than a time at which the nucleic acid molecule normally is expressed, or at a level other than the level at which the nucleic acid molecule normally is expressed. In wild type *Arabidopsis*, for example, AGL8 expression is normally restricted during the later stages of floral development to the carpel valves and is not seen in the replum, which is the small strip of cells separating the carpel valves. However, under control of a constitutive promoter such as the cauliflower mosaic virus 35S promoter, AGL8 is expressed in the replum and, additionally, is expressed at higher than normal levels

in other tissues such as valve margin and, thus, is ectopically expressed.

The term "delayed," as used herein in reference to the timing of seed dispersal in a fruit produced by a non-naturally occurring seed plant of the invention, means a significantly later time of seed dispersal as compared to the time seeds normally are dispersed from a corresponding seed plant lacking an ectopically expressed nucleic acid molecule encoding an AGL8-like gene product. Thus, the term "delayed" is used broadly to encompass both seed dispersal that is significantly postponed as compared to the seed dispersal in a corresponding seed plant, and to seed dispersal that is completely precluded, such that fruits never release their seeds unless there is human or other intervention.

It is recognized that there can be natural variation of the time of seed dispersal within a seed plant species or variety. However, a "delay" in the time of seed dispersal in a non-naturally occurring seed plant of the invention readily can be identified by sampling a population of the non-naturally occurring seed plants and determining that the normal distribution of seed dispersal times is significantly later, on average, than the normal distribution of seed dispersal times in a population of the corresponding seed plant species or variety that does not contain an ectopically expressed nucleic acid molecule encoding an AGL8-like gene product. Thus, production of non-naturally occurring seed plants of the invention provides a means to skew the normal distribution of the time of seed dispersal from pollination, such that seeds are dispersed, on average, at least about 1%, 2%, 5%, 10%, 30%, 50% or 100% later than in the corresponding seed plant species that does

not contain an ectopically expressed nucleic acid molecule encoding an AGL8-like gene product.

A delay in seed dispersal of even one to two days can be valuable in increasing the amount of seed successfully harvested from a seed plant. In canola rapeseed, for example, dehiscence normally occurs about 8 weeks post-pollination. In a non-naturally occurring canola rapeseed that ectopically expresses an AGL8-like gene product, dehiscence can occur one to two days later than in the wild type variety, allowing a significantly greater percentage of the seed crop to be harvested rather than lost through uncontrolled seed dispersal.

The present invention relates to the use of nucleic acid molecules encoding particular "AGAMOUS-LIKE" or "AGL" gene products. AGAMOUS (AG) is a floral organ identity gene, one of a related family of transcription factors that, in various combinations, specify the identity of the floral organs: the petals, sepals, stamens and carpels (Bowman et al., Devel. 112:1-20 (1991); Weigel and Meyerowitz, Cell 78:203-209 (1994); Yanofsky, Annual Rev. Plant Physiol. Mol. Biol. 46:167-188 (1995)). The AGAMOUS gene product is essential for specification of carpel and stamen identity (Bowman et al., The Plant Cell 1:37-52 (1989); Yanofsky et al., Nature 346:35-39 (1990)). Related genes have recently been identified and denoted "AGAMOUS-LIKE" or "AGL" genes (Ma et al., Genes Devel. 5:484-495 (1991); Mandel and Yanofsky, The Plant Cell 7:1763-1771 (1995), which is incorporated herein by reference).

AGL8, like AGAMOUS and other AGL genes, is characterized, in part, in that it is a plant MADS box gene. The plant MADS box genes generally encode proteins



of about 260 amino acids including a highly conserved MADS domain of about 56 amino acids (Riechmann and Meyerowitz, Biol. Chem. 378:1079-1101 (1997), which is incorporated herein by reference). The MADS domain, which was first identified in the *Arabidopsis AGAMOUS* and *Antirrhinum majus DEFICIENS* genes, is conserved among transcription factors found in humans (serum response factor; SRF) and yeast (MCM1; Norman et al., Cell 55:989-1003 (1988); Passmore et al., J. Mol. Biol. 204:593-606 (1988), and is the most highly conserved region of the MADS domain proteins. The MADS domain is the major determinant of sequence specific DNA-binding activity and can also perform dimerization and other accessory functions (Huang et al., The Plant Cell 8:81-94 (1996)). The MADS domain frequently resides at the N-terminus, although some proteins contain additional residues N-terminal to the MADS domain.

The "intervening domain" or "I-domain," located immediately C-terminal to the MADS domain, is a weakly conserved domain having a variable length of approximately 30 amino acids (Purugganan et al., Genetics 140:345-356 (1995)). In some proteins, the I-domain plays a role in the formation of DNA-binding dimers. A third domain present in plant MADS domain proteins is a moderately conserved 70 amino acid region denoted the "keratin-like domain" or "K-domain." Named for its similarity to regions of the keratin molecule, the structure of the K-domain appears capable of forming amphipathic helices and may mediate protein-protein interactions (Ma et al., Genes Devel. 5:484-495 (1991)). The most variable domain, both in sequence and in length, is the carboxy-terminal or "C-domain" of the MADS domain proteins. Dispensable for DNA binding and protein

dimerization in some MADS domain proteins, the function of this C-domain remains unknown.

5 *Arabidopsis* AGL8 is a 242 amino acid MADS box protein (see Figure 6; SEQ ID NO:2; Mandel and Yanofsky, *supra*, 1995). The AGL8 MADS domain resides at amino acids 2 to 56 of SEQ ID NO:2. The K-domain of AGL8 resides at amino acids 92 to 158 of SEQ ID NO:2.

10 In wild-type *Arabidopsis*, AGL8 RNA accumulates in two distinct phases, the first occurring during inflorescence development in the stem and cauline leaves and the second in the later stages of flower development (Mandel and Yanofsky, *supra*, 1995). In particular, AGL8 RNA is first detected in the inflorescence meristem as  
15 soon as the plant switches from vegetative to reproductive development. As the inflorescence stem elongates, AGL8 RNA accumulates in the inflorescence meristem and in the stem. Secondly, although AGL8 is not detected in the initial stages (1 and 2) of flower  
20 development, AGL8 expression resumes at approximately stage 3 in the center of the floral dome in the region corresponding to the fourth (carpel) whorl. AGL8 expression is excluded from all other primordia and the pedicel. The time of AGL8 expression in the fourth  
25 carpel whorl generally corresponds to the time at which the organ identity genes *APETALA3*, *PISTILLATA* AND *AGAMOUS* begin to be expressed (Yanofsky et al., Nature 346:35-39 (1990); Drews et al., Cell 65:991-1002 (1991); Jack et al., Cell 68:683-697 (1992); Goto and Meyerowitz, Genes  
30 Devel. 8:1548-1560 (1994)). At later stages, AGL8 expression becomes localized to the carpel walls, in the region that constitutes the valves of the ovary, and is absent from nearly all other cell types of the carpel. No AGL8 RNA expression is detected in the ovules,

stigmatic tissues or the septum that divides the ovary. Thus, in nature, AGL8 expression during the later stages of floral development is restricted to the valves of the carpels and to the cells within the style.

5           As used herein, the term "AGL8-like gene product" means a gene product that has the same or similar function as *Arabidopsis* AGL8 such that, when ectopically expressed in a seed plant, the normal development of the dehiscence zone is altered, and seed  
10 dispersal is delayed. An AGL8-like gene product can have, for example, the ability to convert cells of the outer replum to a valve cell identity. *Arabidopsis* AGL8 (SEQ ID NO:2) is an example of an AGL8-like gene product as defined herein. As disclosed in Example I, ectopic  
15 expression of *Arabidopsis* AGL8 (SEQ ID NO:2) under control of a tandem CaMV 35S promoter, in which the intrinsic promoter element has been duplicated, alters formation of the dehiscence zone, thereby resulting in fruit characterized by a complete lack of seed dispersal.  
20 An AGL8-like gene product also can be characterized, in part, by its ability to interact with AGL1 and, additionally, its ability to interact with AGL5.

          An AGL8-like gene product generally is characterized, in part, by having an amino acid sequence  
25 that has at least about 50% amino acid identity with the amino acid sequence of *Arabidopsis* AGL8 (SEQ ID NO: 2). An AGL8-like gene product can have, for example, an amino acid sequence with greater than about 65% amino acid sequence identity with *Arabidopsis* AGL8 (SEQ ID NO:2),  
30 preferably greater than about 75% amino acid identity with *Arabidopsis* AGL8 (SEQ ID NO:2), more preferably greater than about 85% amino acid identity with *Arabidopsis* AGL8 (SEQ ID NO:2), and can be a sequence

having greater than about 90%, 95% or 97% amino acid identity with *Arabidopsis* AGL8 (SEQ ID NO:2).

Preferably, an AGL8-like gene product is  
5 orthologous to the seed plant species in which it is ectopically expressed. A nucleic acid molecule encoding *Arabidopsis* AGL8 (SEQ ID NO:2), for example, can be ectopically expressed in an *Arabidopsis* plant to produce a non-naturally occurring *Arabidopsis* variety  
10 characterized by delayed seed dispersal. Similarly, a nucleic acid molecule encoding canola AGL8 can be ectopically expressed in a canola plant to produce a non-naturally occurring canola variety characterized by delayed seed dispersal.

15 A nucleic acid molecule encoding an AGL8-like gene product also can be ectopically expressed in a heterologous seed plant to produce a non-naturally occurring seed plant characterized by delayed seed dispersal. AGAMOUS-like gene products have been widely  
20 conserved throughout the plant kingdom; for example, AGAMOUS has been conserved in tomato (TAG1) and maize (ZAG1), indicating that orthologs of AGAMOUS-like genes are present in most, if not all, angiosperms (Pnueli et al., The Plant Cell 6:163-173 (1994); Schmidt et al., The  
25 Plant Cell 5:729-737 (1993)). AGL8-like gene products such as AGL8 orthologs also can be conserved and can function across species boundaries to delay seed dispersal. Thus, ectopic expression of a nucleic acid molecule encoding *Arabidopsis* AGL8 (SEQ ID NO:2) in a  
30 heterologous seed plant within the *Brassicaceae* such as *Brassica napus* L. (rapeseed) or within the *Fabaceae* such as in *Glycine* (soybean) can alter normal development of the dehiscence zone, thereby resulting in delayed seed dispersal. Furthermore, a nucleic acid molecule encoding

*Arabidopsis* AGL8 (SEQ ID NO:2), for example, can be ectopically expressed in more distantly related heterologous seed plants, including dehiscent seed plants as well as other dicotyledonous and monocotyledonous angiosperms and gymnosperms and, upon ectopic expression, can alter normal development of the dehiscence zone and delay seed dispersal in the heterologous seed plant.

As used herein, the term "AGL8-like gene product" encompasses an active segment of an AGL8-like gene product, which is a polypeptide portion of an AGL8-like gene product that, when ectopically expressed, alters normal development of the dehiscence zone and delays seed dispersal. An active segment can be, for example, an amino terminal, internal or carboxy terminal fragment of *Arabidopsis* AGL8 (SEQ ID NO:2) that, when ectopically expressed in a seed plant, alters normal development of the dehiscence zone and delays seed dispersal. An active segment of an AGL8-like gene product can include, for example, the MADS domain and can have the ability to bind DNA specifically. The skilled artisan will recognize that a nucleic acid molecule encoding an active segment of an AGL8-like gene product can be useful in producing a seed plant of the invention characterized by delayed seed dispersal and in the related methods and kits of the invention described further below.

An active segment of an AGL8-like gene product can be identified using the methods described in Example I or using other routine methodology. Briefly, a seed plant such as *Arabidopsis* can be transformed with a nucleic acid molecule under control of a constitutive regulatory element such as a tandem CaMV 35S promoter. Phenotypic analysis of the seed plant reveals whether a



seed plant ectopically expressing a particular polypeptide portion is characterized by delayed seed dispersal. In transgenic plants in which seed dispersal is delayed, further analysis can be performed to confirm that normal development of the dehiscence zone has been altered. For analysis of a large number of polypeptide portions of an AGL8-like gene product, nucleic acid molecules encoding the polypeptide portions can be assayed in pools, and active pools subsequently subdivided to identify the active nucleic acid molecule.

In one embodiment, the invention provides a non-naturally occurring seed plant that is characterized by delayed seed dispersal due to ectopic expression of a nucleic acid molecule encoding an AGL8-like gene product having substantially the amino acid sequence of an AGL8 ortholog. As used herein, the term "AGL8 ortholog" means an ortholog of *Arabidopsis* AGL8 (SEQ ID NO:2) and refers to an AGL8-like gene product that, in a particular seed plant variety, has the highest percentage homology at the amino acid level to *Arabidopsis* AGL8 (SEQ ID NO:2). An AGL8 ortholog can be, for example, a *Brassica* AGL8 ortholog such as a *Brassica napus* L. AGL8 ortholog, or a *Fabacea* AGL8 ortholog such as a soybean, pea, lentil, or bean AGL8 ortholog. An AGL8 ortholog from the long-day plant *Sinapis alba*, designated SaMADS B, has been described (Menzel et al., Plant J. 9:399-408 (1996), which is incorporated herein by reference). Novel AGL8 ortholog cDNAs can be isolated from additional seed plant species using a nucleotide sequence as a probe and methods well known in the art of molecular biology (Glick and Thompson (eds.), Methods in Plant Molecular Biology and Biotechnology, Boca Raton, FL: CRC Press (1993); Sambrook et al. (eds.), Molecular Cloning: A Laboratory Manual (Second Edition), Plainview, NY: Cold Spring



Harbor Laboratory Press (1989), each of which is incorporated herein by reference).

As used herein, the term "substantially the amino acid sequence," when used in reference to an AGL8 ortholog, is intended to mean a polypeptide or polypeptide segment having an identical amino acid sequence, or a polypeptide or polypeptide segment having a similar, non-identical sequence that is considered by those skilled in the art to be a functionally equivalent amino acid sequence. For example, an AGL8-like gene product having substantially the amino acid sequence of *Arabidopsis* AGL8 can have an amino acid sequence identical to the sequence of *Arabidopsis* AGL8 (SEQ ID NO:2) shown in Figure 6, or a similar, non-identical sequence that is functionally equivalent. In particular, an amino acid sequence that is "substantially the amino acid sequence" of AGL8 can have one or more modifications such as amino acid additions, deletions or substitutions relative to the AGL8 amino acid sequence shown (SEQ ID NO:2), provided that the modified polypeptide retains substantially the ability to alter normal development of the dehiscence zone and delay seed dispersal when ectopically expressed in the seed plant. Comparison of sequences for substantial similarity can be performed between two sequences of any length and usually is performed with sequences between about 6 and 1200 residues, preferably between about 10 and 100 residues and more preferably between about 25 and 35 residues. Such comparisons for substantial similarity are performed using methodology routine in the art.

It is understood that minor modifications of primary amino acid sequence can result in an AGL8-like gene product that has substantially equivalent or

enhanced function as compared to the AGL8 ortholog from which it was derived. Further, various molecules can be attached to an AGL8 ortholog or active segment thereof, for example, other polypeptides, antigenic or other peptide tags, carbohydrates, lipids, or chemical moieties. Such modifications are included within the term AGL8 ortholog as defined herein.

One or more point mutations can be introduced into a nucleic acid molecule encoding an AGL8 ortholog to yield a modified nucleic acid molecule using, for example, site-directed mutagenesis (see Wu (Ed.), Meth. In Enzymol. Vol. 217, San Diego: Academic Press (1993); Higuchi, "Recombinant PCR" in Innis et al. (Ed.), PCR Protocols, San Diego: Academic Press, Inc. (1990), each of which is incorporated herein by reference). Such mutagenesis can be used to introduce a specific, desired amino acid insertion, deletion or substitution; alternatively, a nucleic acid sequence can be synthesized having random nucleotides at one or more predetermined positions to generate random amino acid substitutions. Scanning mutagenesis also can be useful in generating a modified nucleic acid molecule encoding substantially the amino acid sequence of an AGL8 ortholog.

Modified nucleic acid molecules can be routinely assayed for the ability to alter normal development of the dehiscence zone and to delay seed dispersal. In the same manner as described in Examples I and III, a nucleic acid molecule encoding substantially the amino acid sequence of an AGL8 ortholog can be ectopically expressed, for example, using a constitutive regulatory element such as the CaMV 35S promoter or using a dehiscence zone-selective regulatory element such as the AGL1 promoter. If such ectopic expression results in

a seed plant in which the dehiscence zone fails to develop and in which seed dispersal is delayed, the modified polypeptide or segment is an "AGL8 ortholog" as defined herein.

5                   A non-naturally occurring seed plant of the invention that is characterized by delayed seed dispersal can be one of a variety of seed plant species, such as a dehiscent seed plant or another monocotyledonous and dicotyledonous angiosperm or gymnosperm. A useful seed  
10 plant of the invention can be a dehiscent seed plant, and a particularly useful seed plant of the invention can be a member of the *Brassicaceae*, such as rapeseed, or a member of the *Fabaceae*, such as a soybean, pea, lentil or bean plant.

15                   As used herein, the term "seed plant" means an angiosperm or gymnosperm. An angiosperm is a seed-bearing plant whose seeds are borne in a mature ovary (fruit). An angiosperm commonly is recognized as a flowering plant. Angiosperms are divided into two broad  
20 classes based on the number of cotyledons, which are seed leaves that generally store or absorb food. Thus, a monocotyledonous angiosperm is an angiosperm having a single cotyledon, whereas a dicotyledonous angiosperm is an angiosperm having two cotyledons. A variety of  
25 angiosperms are known including, for example, oilseed plants, leguminous plants, fruit-bearing plants, ornamental flowers, cereal plants and hardwood trees, which general classes are not necessarily exclusive. The skilled artisan will recognize that the methods of the  
30 invention can be practiced using these or other angiosperms, as desired. A gymnosperm is a seed-bearing plant with seeds not enclosed in an ovary.

In one embodiment, the invention provides a non-naturally occurring dehiscent seed plant that is characterized by delayed seed dispersal due to ectopic expression of a nucleic acid molecule encoding an AGL8-like gene product in the dehiscent seed plant. As used herein, the term "dehiscent seed plant" means a seed plant that produces a dry dehiscent fruit, which has fruit walls that open to permit escape of the seeds contained therein. Dehiscent fruits commonly contain several seeds and include the fruits known, for example, as legumes, capsules and siliques.

In one embodiment, the invention provides a non-naturally occurring seed plant that is characterized by delayed seed dispersal due to ectopic expression of a nucleic acid molecule encoding an AGL8-like gene product, where the seed plant is a member of the Brassicaceae. The Brassicaceae, commonly known as the Brassicas, are a diverse group of crop plants with great economic value worldwide (see, for example, Williams and Hill, Science 232:1385-1389 (1986), which is incorporated herein by reference). The Brassicaceae produce seed oils for margarine, salad oil, cooking oil, plastic and industrial uses; condiment mustard; leafy, stored, processed and pickled vegetables; animal fodders and green manures for soil rejuvenation. A particularly useful non-naturally occurring Brassica seed plant of the invention is the oilseed plant canola.

There are six major Brassica species of economic importance, each containing a range of plant forms. *Brassica napus* includes plants such as the oilseed rapes and rutabaga. *Brassica oleracea* are the cole crops such as cabbage, cauliflower, kale, kohlrabi and Brussels sprouts. *Brassica campestris* (*Brassica*

rapa) includes plants such as Chinese cabbage, turnip and pak choi. *Brassica juncea* includes a variety of mustards; *Brassica nigra* is the black mustard; and *Brassica carinata* is Ethiopian mustard. The skilled artisan understands that any member of the *Brassicaceae* can be modified as disclosed herein to produce a non-naturally occurring *Brassica* plant characterized by delayed seed dispersal.

In a second embodiment, the invention provides a non-naturally occurring seed plant that is characterized by delayed seed dispersal due to ectopic expression of a nucleic acid molecule encoding an AGL8-like gene product, where the seed plant is a member of the *Fabaceae*. The *Fabaceae*, which are commonly known as members of the pea family, are seed plants that produce a characteristic dry dehiscent fruit known as a legume. The legume is derived from a single carpel and dehisces along the suture of the carpel margins and along the median vein. The *Fabaceae* encompass both grain legumes and forage legumes. Grain legumes include, for example, soybean (*glycine*), pea, chickpea, moth bean, broad bean, kidney bean, lima bean, lentil, cowpea, dry bean and peanut. Forage legumes include alfalfa, lucerne, birdsfoot trefoil, clover, *stylosanthes* species, *lotononis bainesii* and sainfoin. The skilled artisan will recognize that any member of the *Fabaceae* can be modified as disclosed herein to produce a non-naturally occurring seed plant of the invention characterized by delayed seed dispersal.

A non-naturally occurring seed plant of the invention characterized by delayed seed dispersal also can be a member of the plant genus *Cuphea* (family *Lythraceae*). A *Cuphea* seed plant is particularly

valuable since Cuphea oilseeds contain industrially and nutritionally important medium-chain fatty acids, especially lauric acid, which is currently supplied only by coconut and palm kernel oils.

5           A non-naturally occurring seed plant of the invention also can be, for example, one of the monocotyledonous grasses, which produce many of the valuable small-grain cereal crops of the world. In a non-naturally occurring small grain cereal plant of the  
10 invention, grain remains on the seed plant longer and, Ectopic expression of a nucleic acid molecule encoding an AGL8-like gene product, or suppression of AGL1 and AGL5 expression as described below, can be useful in generating a non-naturally occurring small grain cereal  
15 plant, such as a barley, wheat, oat, rye, orchard grass, guinea grass, sorghum or turf grass plant characterized by delayed seed dispersal.

          The invention also provides a transgenic seed plant that is characterized by delayed seed dispersal due  
20 to ectopic expression of a nucleic acid molecule encoding an AGL8-like gene product. In a transgenic seed plant of the invention, the ectopically expressed nucleic acid molecule encoding an AGL8-like gene product can be operatively linked to an exogenous regulatory element.  
25 The invention provides, for example, a transgenic seed plant characterized by delayed seed dispersal having an ectopically expressed nucleic acid molecule encoding an AGL8-like gene product that is operatively linked to an exogenous constitutive regulatory element. In one  
30 embodiment, the invention provides a transgenic seed plant that is characterized by delayed seed dispersal due to ectopic expression of an exogenous nucleic acid molecule encoding substantially the amino acid sequence



of an AGL8 ortholog operatively linked to an exogenous cauliflower mosaic virus 35S promoter.

The invention also provides a transgenic seed plant that is characterized by delayed seed dispersal due to ectopic expression of a nucleic acid molecule encoding an AGL8-like gene product operatively linked to a dehiscence zone-selective regulatory element. The dehiscence zone-selective regulatory element can be, for example, an *AGL1* regulatory element or *AGL5* regulatory element. The *AGL1* regulatory element can be derived from the *Arabidopsis* *AGL1* genomic sequence disclosed herein as SEQ ID NO:3 and can be, for example, a 5' regulatory sequence or intronic regulatory element. Similarly, the *AGL5* regulatory element can be derived from the *Arabidopsis* *AGL5* genomic sequence disclosed herein as SEQ ID NO:4 and can be, for example, a 5' regulatory sequence or intronic regulatory element.

In one embodiment, a transgenic seed plant of the invention has an ectopically expressed exogenous nucleic acid molecule encoding substantially the amino acid sequence of an AGL8 ortholog operatively linked to a dehiscence zone-selective regulatory element that is an *AGL1* regulatory element having at least fifteen contiguous nucleotides of nucleotides 1 to 2599 of SEQ ID NO:3; nucleotides 2833 to 4128 of SEQ ID NO:3; nucleotides 4211 to 4363 of SEQ ID NO:3; nucleotides 4426 to 4554 of SEQ ID NO:3; nucleotides 4796 to 4878 of SEQ ID NO:3; nucleotides 4921 to 5028 of SEQ ID NO:3; or nucleotides 5421 to 5682 of SEQ ID NO:3.

In another embodiment, a transgenic seed plant of the invention has an ectopically expressed exogenous nucleic acid molecule encoding substantially the amino

acid sequence of an AGL8 ortholog operatively linked to a dehiscence zone-selective regulatory element that is an AGL5 regulatory element having at least fifteen contiguous nucleotides of nucleotides 1 to 1890 of SEQ ID NO:4; nucleotides 2536 to 2683 of SEQ ID NO:4; nucleotides 2928 to 5002 of SEQ ID NO:4; nucleotides 5085 to 5204 of SEQ ID NO:4; nucleotides 5367 to 5453 of SEQ ID NO:4; nucleotides 5645 to 5734 of SEQ ID NO:4; or nucleotides 6062 to 6138 of SEQ ID NO:4.

10           As used herein, the term "transgenic" refers to a seed plant that contains an exogenous nucleic acid molecule, which can be derived from the same seed plant species or a heterologous seed plant species.

          The term "exogenous," as used herein in reference to a nucleic acid molecule and a transgenic seed plant, means a nucleic acid molecule originating from outside the seed plant. An exogenous nucleic acid molecule can be, for example, a nucleic acid molecule encoding an AGL8-like gene product or an exogenous regulatory element such as a constitutive regulatory element or a dehiscence zone-selective regulatory element, as described further below. An exogenous nucleic acid molecule can have a naturally occurring or non-naturally occurring nucleotide sequence and can be a heterologous nucleic acid molecule derived from a different seed plant species than the seed plant into which the nucleic acid molecule is introduced or can be a nucleic acid molecule derived from the same seed plant species as the seed plant into which it is introduced.

30           The term "operatively linked," as used in reference to a regulatory element and a nucleic acid molecule, means that the regulatory element confers

regulated expression upon the operatively linked nucleic acid molecule. Thus, the term "operatively linked," as used in reference to an exogenous regulatory element such as a dehiscence zone-selective regulatory element and a nucleic acid molecule encoding an AGL8-like gene product, means that the dehiscence zone-selective regulatory element is linked to the nucleic acid molecule encoding an AGL8-like gene product such that the expression pattern of the dehiscence zone-selective regulatory element is conferred upon the nucleic acid molecule encoding the AGL8-like gene product. It is recognized that a regulatory element and a nucleic acid molecule that are operatively linked have, at a minimum, all elements essential for transcription, including, for example, a TATA box.

As used herein, the term "constitutive regulatory element" means a regulatory element that confers a level of expression upon an operatively linked nucleic molecule that is relatively independent of the cell or tissue type in which the constitutive regulatory element is expressed. A constitutive regulatory element that is expressed in a seed plant generally is widely expressed in a large number of cell and tissue types.

A variety of constitutive regulatory elements useful for ectopic expression in a transgenic seed plant are well known in the art. The cauliflower mosaic virus 35S (CaMV 35S) promoter, for example, is a well-characterized constitutive regulatory element that produces a high level of expression in all plant tissues (Odell et al., Nature 313:810-812 (1985)). The CaMV 35S promoter can be particularly useful due to its activity in numerous diverse seed plant species (Benfey and Chua, Science 250:959-966 (1990); Futterer et al., Physiol.

Plant 79:154 (1990); Odell et al., *supra*, 1985). A tandem 35S promoter, in which the intrinsic promoter element has been duplicated, confers higher expression levels in comparison to the unmodified 35S promoter (Kay et al., Science 236:1299 (1987)). Other constitutive regulatory elements useful for ectopically expressing a nucleic acid molecule encoding an AGL8-like gene product in a transgenic seed plant of the invention include, for example, the cauliflower mosaic virus 19S promoter; the Figwort mosaic virus promoter; and the nopaline synthase (nos) gene promoter (Singer et al., Plant Mol. Biol. 14:433 (1990); An, Plant Physiol. 81:86 (1986)).

Additional constitutive regulatory elements including those for efficient ectopic expression in monocots also are known in the art, for example, the pEmu promoter and promoters based on the rice Actin-1 5' region (Last et al., Theor. Appl. Genet. 81:581 (1991); Mcelroy et al., Mol. Gen. Genet. 231:150 (1991); Mcelroy et al., Plant Cell 2:163 (1990)). Chimeric regulatory elements, which combine elements from different genes, also can be useful for ectopically expressing a nucleic acid molecule encoding an AGL8-like gene product (Comai et al., Plant Mol. Biol. 15:373 (1990)). One skilled in the art understands that a particular constitutive regulatory element is chosen based, in part, on the seed plant species in which a nucleic acid molecule encoding an AGL8-like gene product is to be ectopically expressed and on the desired level of expression.

30 An exogenous regulatory element useful in a transgenic seed plant of the invention also can be an inducible regulatory element, which is a regulatory element that confers conditional expression upon an

operatively linked nucleic acid molecule, where expression of the operatively linked nucleic acid molecule is increased in the presence of a particular inducing agent or stimulus as compared to expression of the nucleic acid molecule in the absence of the inducing agent or stimulus. Particularly useful inducible regulatory elements include copper-inducible regulatory elements (Mett et al., Proc. Natl. Acad. Sci. USA 90:4567-4571 (1993); Furst et al., Cell 55:705-717 (1988)); tetracycline and chlor-tetracycline-inducible regulatory elements (Gatz et al., Plant J. 2:397-404 (1992); Röder et al., Mol. Gen. Genet. 243:32-38 (1994); Gatz, Meth. Cell Biol. 50:411-424 (1995)); ecdysone inducible regulatory elements (Christopherson et al., Proc. Natl. Acad. Sci. USA 89:6314-6318 (1992); Kreutzweiser et al., Ecotoxicol. Environ. Safety 28:14-24 (1994)); heat shock inducible regulatory elements (Takahashi et al., Plant Physiol. 99:383-390 (1992); Yabe et al., Plant Cell Physiol. 35:1207-1219 (1994); Ueda et al., Mol. Gen. Genet. 250:533-539 (1996)); and lac operon elements, which are used in combination with a constitutively expressed lac repressor to confer, for example, IPTG-inducible expression (Wilde et al., EMBO J. 11:1251-1259 (1992)).

25           An inducible regulatory element useful in the transgenic seed plants of the invention also can be, for example, a nitrate-inducible promoter derived from the spinach nitrite reductase gene (Back et al., Plant Mol. Biol. 17:9 (1991)) or a light-inducible promoter, such as  
30           that associated with the small subunit of RuBP carboxylase or the LHCP gene families (Feinbaum et al., Mol. Gen. Genet. 226:449 (1991); Lam and Chua, Science 248:471 (1990)). Additional inducible regulatory elements include salicylic acid inducible regulatory

elements (Uknes et al., Plant Cell 5:159-169 (1993); Bi  
et al., Plant J. 8:235-245 (1995)); plant  
hormone-inducible regulatory elements  
(Yamaguchi-Shinozaki et al., Plant Mol. Biol. 15:905  
5 (1990); Kares et al., Plant Mol. Biol. 15:225 (1990));  
and human hormone-inducible regulatory elements such as  
the human glucocorticoid response element (Schena et al.,  
Proc. Natl. Acad. Sci. USA 88:10421 (1991)).

It should be recognized that a non-naturally  
10 occurring seed plant of the invention, which contains an  
ectopically expressed nucleic acid molecule encoding an  
AGL8-like gene product, also can contain one or more  
additional modifications, including naturally and  
non-naturally occurring modifications, that can modulate  
15 the delay in seed dispersal. For example, the plant  
hormone ethylene promotes fruit dehiscence, and modified  
expression or activity of positive or negative regulators  
of the ethylene response can be included in a seed plant  
of the invention (see, generally, Meakin and Roberts, J.  
20 Exp. Botany 41:1003-1011 (1990); Ecker, Science  
268:667-675 (1995); Chao et al., Cell 89:1133-1144  
(1997)).

Mutations in positive regulators of the  
ethylene response show a reduction or absence of  
25 responsiveness to treatment with exogenous ethylene.  
*Arabidopsis* mutations in positive regulators of the  
ethylene response include mutations in *etr*, which  
inactivate a histidine kinase ethylene receptor (Bleeker  
et al., Science 241:1086-1089 (1988); Schaller and  
30 Bleeker, Science 270:1809-1811 (1995)); *ers* (Hua et al.,  
Science 269:1712-1714 (1995)); *ein2* (Guzman and Ecker,  
Plant Cell 2:513 (1990)); *ein3* (Rothenberg and Ecker,  
Sem. Dev. Biol. Plant Dev. Genet. 4:3-13 (1993); Kieber



and Ecker, Trends Genet. 9:356-362 (1993)); *ain1* (van der Straeten et al., Plant Physiol. 102:401-408 (1993)); *eti* (Harpham et al., An. Bot. 68:55 (1991)) and *ein4*, *ein5*, *ein6*, and *ein7* (Roman et al., Genetics 139: 1393-1409  
5 (1995)). Similar genetic functions are found in other seed plant species; for example, the *never-ripe* mutation corresponds to *etr* and confers ethylene insensitivity in tomato (Lanahan et al., The Plant Cell 6:521-530 (1994); Wilkinson et al., Science 270:1807-1809 (1995)). A seed  
10 plant of the invention can include a modification that results in altered expression or activity of any such positive regulator of the ethylene response. A mutation in a positive regulator, for example, can be included in a seed plant of the invention and can modify the delay in  
15 seed dispersal in such plants, for example, by further postponing the delay in seed dispersal.

Mutations in negative regulators of the ethylene response display ethylene responsiveness in the absence of exogenous ethylene. Such mutations include  
20 those relating to ethylene overproduction, for example, the *eto1*, *eto2*, and *eto3* mutants, and those relating to constitutive activation of the ethylene signalling pathway, for example, mutations in *CTR1*, a negative regulator with sequence similarity to the Raf family of  
25 protein kinases (Kieber et al., Cell 72:427-441 (1993), which is incorporated herein by reference). A seed plant of the invention can include a modification that results in altered expression or activity of any such negative  
30 regulator of the ethylene response. A mutation resulting in ethylene responsiveness in the absence of exogenous ethylene, for example, can be included in a non-naturally occurring seed plant of the invention and can modify, for example, diminish, the delay in seed dispersal.

Fruit morphological mutations also can be included in a seed plant of the invention. Such mutations include those in carpel identity genes such as *AGAMOUS* (Bowman et al., *supra*, 1989; Yanofsky et al., *supra*, 1990) and in genes required for normal fruit development such as *ETTIN*, *CRABS CLAW*, *SPATULA*, *AGL8* and *TOUSLED* (Sessions et al., *Development* 121:1519-1532 (1995); Alvarez and Smyth, *Flowering Newsletter* 23:12-17 (1997); and Roe et al., *Cell* 75:939-950 (1993)). Thus, it is understood that a seed plant of the invention having an ectopically expressed nucleic acid molecule encoding an AGL8-like gene product can include one or more additional genetic modifications, which can diminish or enhance the delay in seed dispersal.

The present invention also provides methods of producing a non-naturally occurring seed plant characterized by delayed seed dispersal. A method of the invention entails ectopically expressing a nucleic acid molecule encoding an AGL8-like gene product in the seed plant, whereby seed dispersal is delayed due to ectopic expression of the nucleic acid molecule.

As discussed above, the term "ectopically" refers to expression of a nucleic acid molecule encoding an AGL8-like gene product in a cell type other than a cell type in which the nucleic acid molecule is normally expressed, at a time other than a time at which the nucleic acid molecule is normally expressed or at an expression level other than the level at which the nucleic acid normally is expressed. In wild type *Arabidopsis*, for example, AGL8 expression is normally restricted during the later stages of floral development to the carpel valves and is not seen in the outer replum. In the methods of the invention, particularly useful

ectopic expression of a nucleic acid molecule encoding an AGL8-like gene product involves expression in the cells of the outer replum, which are the progenitors of the dehiscence zone.

5           Actual ectopic expression of an AGL8-like gene product is dependent on various factors. The ectopic expression can be widespread expression throughout most or all plant tissues or can be expression restricted to a small number of plant tissues, and can be achieved by a  
10           variety of routine techniques. Mutagenesis, including seed or pollen mutagenesis, can be used to generate a non-naturally occurring seed plant, in which a nucleic acid molecule encoding an AGL8-like gene product is ectopically expressed. Ethylmethane sulfonate (EMS)  
15           mutagenesis, transposon mediated mutagenesis or T-DNA mediated mutagenesis also can be useful in ectopically expressing an AGL8-like gene product to produce a seed plant characterized by delayed seed dispersal (see, generally, Glick and Thompson, *supra*, 1993). While not  
20           wishing to be bound by any particular mechanism, ectopic expression in a mutagenized plant can result from inactivation of one or more negative regulators of AGL8, for example, from the combined inactivation of AGL1 and AGL5.

25           Ectopic expression of an AGL8-like gene product also can be achieved by expression of a nucleic acid encoding an AGL8-like gene product from a heterologous regulatory element or from a modified variant of its own promoter. Heterologous regulatory elements include  
30           constitutive regulatory elements, which result in expression of the AGL8-like gene product in the outer replum as well as in a variety of other cell types, and dehiscence zone-selective regulatory elements, which

produce selective expression of an AGL8-like gene product in a limited number of cell types including the cells of the valve margin or the dehiscence zone.

Ectopic expression of a nucleic acid molecule  
5 encoding an AGL8-like gene product can be achieved using an endogenous or exogenous nucleic acid molecule encoding an AGL8-like gene product. A recombinant exogenous nucleic acid molecule can contain a heterologous regulatory element that is operatively linked to a  
10 nucleic acid sequence encoding an AGL8-like gene product. Methods for producing the desired recombinant nucleic acid molecule under control of a heterologous regulatory element and for producing a non-naturally occurring seed plant of the invention are well known in the art (see,  
15 generally, Sambrook et al., *supra*, 1989; Glick and Thompson, *supra*, 1993).

An exogenous nucleic acid molecule can be introduced into a seed plant for ectopic expression using a variety of transformation methodologies including  
20 *Agrobacterium*-mediated transformation and direct gene transfer methods such as electroporation and microprojectile-mediated transformation (see, generally, Wang et al. (eds), Transformation of Plants and Soil Microorganisms, Cambridge, UK: University Press (1995),  
25 which is incorporated herein by reference). Transformation methods based upon the soil bacterium *Agrobacterium tumefaciens* are particularly useful for introducing an exogenous nucleic acid molecule into a seed plant. The wild type form of *Agrobacterium* contains  
30 a Ti (tumor-inducing) plasmid that directs production of tumorigenic crown gall growth on host plants. Transfer of the tumor-inducing T-DNA region of the Ti plasmid to a plant genome requires the Ti plasmid-encoded virulence

genes as well as T-DNA borders, which are a set of direct DNA repeats that delineate the region to be transferred. An Agrobacterium-based vector is a modified form of a Ti plasmid, in which the tumor inducing functions are replaced by the nucleic acid sequence of interest to be introduced into the plant host.

Agrobacterium-mediated transformation generally employs cointegrate vectors or, preferably, binary vector systems, in which the components of the Ti plasmid are divided between a helper vector, which resides permanently in the Agrobacterium host and carries the virulence genes, and a shuttle vector, which contains the gene of interest bounded by T-DNA sequences. A variety of binary vectors are well known in the art and are commercially available, for example, from Clontech (Palo Alto, CA). Methods of coculturing Agrobacterium with cultured plant cells or wounded tissue such as leaf tubers, for example, hypocotyledons, stem pieces or (Glick and Thompson, supra, 1993). Wounded cells within the plant tissue that have been infected by Agrobacterium can develop organs de novo when cultured under the appropriate conditions; the resulting transgenic shoots eventually give rise to transgenic plants that ectopically express a nucleic acid molecule encoding an AGL8-like gene product. Agrobacterium also can be used for transformation of whole seed plants as described in Bechtold et al., C.R. Acad. Sci. Paris, Life Sci. 316:1194-1199 (1993), which is incorporated herein by reference). Agrobacterium-mediated transformation is useful for producing a variety of transgenic seed plants (Wang et al., supra, 1995) including transgenic plants of the Brassicaceae family, such as rapeseed, Arabidopsis,

mustard, and flax, and transgenic plants of the *Fabaceae* family such as soybean, pea, lentil and bean.

Microprojectile-mediated transformation also can be used to produce a transgenic seed plant that  
5 ectopically expresses an AGL8-like gene product. This method, first described by Klein et al. (Nature 327:70-73 (1987), which is incorporated herein by reference), relies on microprojectiles such as gold or tungsten that are coated with the desired nucleic acid molecule by  
10 precipitation with calcium chloride, spermidine or PEG. The microprojectile particles are accelerated at high speed into an angiosperm tissue using a device such as the BIOLISTIC PD-1000 (Biorad; Hercules CA).

Microprojectile-mediated delivery or "particle  
15 bombardment" is especially useful to transform seed plants that are difficult to transform or regenerate using other methods. Microprojectile-mediated transformation has been used, for example, to generate a variety of transgenic plant species, including cotton,  
20 tobacco, corn, hybrid poplar and papaya (see Glick and Thompson, *supra*, 1993) as well as cereal crops such as wheat, oat, barley, sorghum and rice (Duan et al., Nature Biotech. 14:494-498 (1996); Shimamoto, Curr. Opin. Biotech. 5:158-162 (1994), each of which is incorporated  
25 herein by reference). In view of the above, the skilled artisan will recognize that *Agrobacterium*-mediated or microprojectile-mediated transformation, as disclosed herein, or other methods known in the art can be used to introduce a nucleic acid molecule encoding an AGL8-like  
30 gene product into a seed plant for ectopic expression.

In another embodiment, the invention provides a non-naturally occurring seed plant that is characterized



by delayed seed dispersal due to suppression of both AGL1 expression and AGL5 expression in the seed plant. Such a non-naturally occurring seed plant characterized by delayed seed dispersal can be, for example, an *agl1 agl5* double mutant.

As disclosed herein, loss-of-function mutations in the *AGL1* and *AGL5* genes were produced by a combination of homologous recombination and disruptive T-DNA insertion (see Example II). Neither *AGL1* nor *AGL5* RNA was expressed in the resulting *agl1 agl5* double mutant, and scanning electron microscopy revealed that the dehiscence zone failed to develop normally in these mutant seed plants. Furthermore, the mature fruits of these seed plants failed to undergo dehiscence, as shown in Figure 5. These results indicate that *AGL1* or *AGL5* gene expression is required for normal development of the dehiscence zone and that suppression of *AGL1* expression combined with suppression of *AGL5* expression in the seed plant can delay dehiscence, allowing the process of pod shatter to be controlled.

The *Arabidopsis* *AGL1* and *AGL5* genes encode MADS box proteins with 85% identity at the amino acid level (see Tables 1 and 2). The *AGL1* and *AGL5* RNA expression patterns also are strikingly similar. In particular, both RNAs are specifically expressed in flowers, where they accumulate in developing carpels. In particular, strong expression of these genes is observed in the outer replum along the valve/replum boundary (Ma et al., *supra*, 1991; Savidge et al., The Plant Cell 7:721-723 (1995); Flanagan et al., The Plant Journal 10:343-353 (1996), each of which is incorporated herein by reference). Thus, *AGL1* and *AGL5* are expressed in the valve margin, at least within the cells of the outer replum.

Table 1						
Amino acid identity in the MADS domain and K-domain of AGAMOUS, AGL1 and AGL5						
	AGAMOUS		AGL1		AGL5	
	MADS	K	MADS	K	MADS	K
AGAMOUS	--	--	95%	68%	95%	62%
AGL1	--	--	--	--	100%	92%
AGL5	--	--	--	--	--	--

Table 2						
Amino acid identity in the I-domain and C-domain of AGAMOUS, AGL1 and AGL5						
	AGAMOUS		AGL1		AGL5	
	I	C	I	C	I	C
AGAMOUS	--	--	--	--	--	--
AGL1	71%	39%	--	--	--	--
AGL5	65%	37%	95%	72%	--	--

As used herein, the term "AGL1" refers to *Arabidopsis* AGL1 (SEQ ID NO:6) or an ortholog of *Arabidopsis* AGL1 (SEQ ID NO:6). An AGL1 ortholog is a MADS box gene product expressed, at least in part, in the valve margins of a seed plant and having homology to the amino acid sequence of *Arabidopsis* AGL1 (SEQ ID NO:6). AGL1 or an AGL1 ortholog can function, in part, by forming a complex with an AGL8-like gene product. An AGL1 ortholog generally has an amino acid sequence having at least about 63% amino acid identity with *Arabidopsis* AGL1 (SEQ ID NO:6) and includes polypeptides having greater than about 70%, 75%, 85% or 95% amino acid identity with *Arabidopsis* AGL1 (SEQ ID NO:6). Given the

close relatedness of the AGL1 and AGL5 gene products, one skilled in the art will recognize that an AGL1 ortholog can be distinguished from an AGL5 ortholog by being more closely related to *Arabidopsis* AGL1 (SEQ ID NO:6) than to  
5 *Arabidopsis* AGL5 (SEQ ID NO:8). An AGL1 ortholog can function in wild type plants, like *Arabidopsis* AGL1, to limit the domain of AGL8-like gene product expression to the carpel valves during the later stages of floral development.

10 As used herein, the term "AGL5" refers to *Arabidopsis* AGL5 (SEQ ID NO:8) or to an ortholog of *Arabidopsis* AGL5 (SEQ ID NO:8). An AGL5 ortholog is a MADS box gene product expressed, at least in part, in the valve margins of a seed plant and having homology to the  
15 amino acid sequence of *Arabidopsis* AGL5 (SEQ ID NO:8). AGL5 or an AGL5 ortholog can function, in part, by forming a complex with an AGL8-like gene product as shown in Example IV. An AGL5 ortholog generally has an amino acid sequence having at least about 60% amino acid  
20 identity with *Arabidopsis* AGL5 (SEQ ID NO:8) and includes polypeptides having greater than about 65%, 70%, 75%, 85% or 95% amino acid identity with *Arabidopsis* AGL5 (SEQ ID NO:8). Given the close relatedness of the AGL1 and AGL5 gene products, one skilled in the art will recognize that  
25 an AGL5 ortholog can be distinguished from an AGL1 ortholog by being more closely related to *Arabidopsis* AGL5 (SEQ ID NO:8) than to *Arabidopsis* AGL1 (SEQ ID NO:6). An AGL5 ortholog can function in wild type plants, like *Arabidopsis* AGL5, to limit the domain of  
30 AGL8-like gene product expression to the carpel valves during the later stages of floral development.

The term "suppressed," as used herein in reference to AGL1 expression, means that the amount of

functional AGL1 protein is reduced in a seed plant in comparison with the amount of functional AGL1 protein in the corresponding wild type seed plant. Similarly, when used in reference to AGL5 expression, the term suppressed  
5 means that the amount of functional AGL5 protein is reduced in a seed plant in comparison with the amount of functional AGL5 protein in the corresponding wild type seed plant. Thus, the term "suppressed," as used herein, encompasses the absence of AGL1 or AGL5 protein in a seed  
10 plant, as well as protein expression that is present but reduced as compared to the level of AGL1 or AGL5 protein expression in a wild type seed plant. Furthermore, the term suppressed refers to AGL1 or AGL5 protein expression that is reduced throughout the entire domain of AGL1 or  
15 AGL5 expression, or to expression that is reduced in some part of the AGL1 or AGL5 expression domain, provided that the resulting seed plant is characterized by delayed seed dispersal.

As used herein, the term "suppressed" also  
20 encompasses an amount of AGL1 or AGL5 protein that is equivalent to wild type AGL1 or AGL5 expression, but where the AGL1 or AGL5 protein has a reduced level of activity. As discussed above, AGL1 and AGL5 each contain a conserved MADS domain; point mutations or gross  
25 deletions within the MADS domain that reduce the DNA-binding activity of AGL1 or AGL5 can reduce or destroy the activity of AGL1 or AGL5 and, therefore, "suppress" AGL1 or AGL5 expression as defined herein. One skilled in the art will recognize that, preferably,  
30 AGL1 expression is essentially absent in the valve margin of a seed plant or the AGL1 protein is essentially non-functional and, similarly, that, preferably, AGL5 expression is essentially absent in the valve margin of

the seed plant or the AGL5 protein is essentially non-functional.

A variety of methodologies can be used to suppress AGL1 or AGL5 expression in a seed plant.

5 Suppression can be achieved by directly modifying the AGL1 or AGL5 genomic locus, for example, by modifying an AGL1 or AGL5 regulatory sequence such that transcription or translation from the AGL1 or AGL5 locus is reduced, or by modifying an AGL1 or AGL5 coding sequence such that  
10 non-functional AGL1 or AGL5 protein is produced. Suppression of AGL1 or AGL5 expression in a seed plant also can be achieved indirectly, for example, by modifying the expression or activity of a protein that regulates AGL1 or AGL5 expression. Methodologies for  
15 effecting suppression of AGL1 or AGL5 expression in a seed plant include, for example, homologous recombination, chemical and transposon-mediated mutagenesis, cosuppression and antisense-based techniques and dominant negative methodologies.

20 Homologous recombination of AGL1 or AGL5 can be used to suppress AGL1 or AGL5 expression in a seed plant as described in Kempin et al., Nature 389:802-803 (1997), which is incorporated herein by reference. Homologous recombination can be used, for example, to replace the  
25 wild type AGL5 genomic sequence with a construct in which the gene for kanamycin resistance is flanked by at least about 1 kb of AGL5 sequence. The use of homologous recombination to suppress AGL5 expression is set forth in Example II.

30 Suppression of AGL1 or AGL5 expression also can be achieved by producing a loss-of-function mutation using transposon-mediated insertional mutagenesis with Ds

transposons or Stm transposons (see, for example, Sundaresan et al., Genes Devel. 9:1797-1810 (1995), which is incorporated herein by reference). Insertion of a transposon into an *AGL1* or *AGL5* target gene can be  
5 identified, for example, by restriction mapping, which can identify the presence of an insertion in the gene promoter or in the coding region, such that expression of functional gene product is suppressed. Insertion of a transposon also can be identified by detecting an absence  
10 of the mRNA encoded by the target gene or by the detecting the absence of the gene product in valve margin. Suppression of *AGL1* or *AGL5* expression also can be achieved by producing a loss-of-function mutation using T-DNA-mediated insertional mutagenesis (see Krysan  
15 et al., Proc. Natl. Acad. Sci., USA 93:8145-8150 (1996)). The use of T-DNA-mediated insertional mutagenesis to suppress *AGL1* expression is disclosed in Example II.

Suppression of *AGL1* or *AGL5* expression in a seed plant also can be achieved using cosuppression,  
20 which is a well known methodology that relies on expression of a nucleic acid molecule in the sense orientation to produce coordinate silencing of the introduced nucleic acid molecule and the homologous endogenous gene (see, for example, Flavell, Proc. Natl.  
25 Acad. Sci., USA 91:3490-3496 (1994); Kooter and Mol, Current Opin. Biol. 4:166-171 (1993), each of which is incorporated herein by reference). Cosuppression is induced most strongly by a large number of transgene copies or by overexpression of transgene RNA and can be  
30 enhanced by modification of the transgene such that it fails to be translated.

Antisense nucleic acid molecules encoding *AGL1* and *AGL5* gene products, or fragments thereof, also can be



used to suppress expression of AGL1 and AGL5 in a seed plant. Antisense nucleic acid molecules reduce mRNA translation or increase mRNA degradation, thereby suppressing gene expression (see, for example, Kooter and Mol, *supra*, 1993; Pnueli et al., The Plant Cell Vol. 6, 175-186 (1994), which is incorporated herein by reference).

To produce a non-naturally occurring seed plant of the invention, in which AGL1 and AGL5 expression each are suppressed, the one or more sense or antisense nucleic acid molecules can be expressed under control of a strong regulatory element that is expressed, at least in part, in the valve margin of the seed plant. The constitutive CaMV 35S promoter (Odell et al., *supra*, 1985), for example, or other constitutive promoters as disclosed herein, can be useful in the methods of the invention. Dehiscence zone-selective regulatory elements also can be useful for expressing one or more sense or antisense nucleic acid molecules in order to suppress AGL1 and AGL5 expression in a seed plant

The skilled artisan will recognize that effective suppression of endogenous AGL1 and AGL5 gene expression depends upon the one or more introduced nucleic acid molecules having a high percentage of homology with the corresponding endogenous gene loci. Nucleic acid molecules encoding *Arabidopsis* AGL1 (SEQ ID NO:5) and AGL5 (SEQ ID NO:7) are provided herein (see, also, Ma et al., *supra*, 1991). Nucleic acid molecules encoding *Arabidopsis* AGL1 and AGL5 can be useful in the methods of the invention or for isolating orthologous AGL1 and AGL5 sequences.

The homology requirement for effective suppression using homologous recombination, cosuppression or antisense methodology can be determined empirically. In general, a minimum of about 80-90% nucleic acid sequence identity is preferred for effective suppression of AGL1 or AGL5 expression. Thus, a nucleic acid molecule encoding a gene ortholog from the family or genus of the seed plant species into which the nucleic acid molecule is to be introduced is preferred for generating the non-naturally occurring seed plants of the invention using homologous recombination, cosuppression or antisense technology. More preferably, a nucleic acid molecule encoding a gene ortholog from the same seed plant species is used for suppressing AGL1 expression and AGL5 expression in a seed plant of the invention. For example, nucleic acid molecules encoding canola AGL1 and AGL5 are preferable for suppressing AGL1 and AGL5 expression in a canola plant.

Although use of a highly homologous nucleic acid molecule is preferred in the methods of the invention, the nucleic acid molecule to be used for homologous recombination, cosuppression or antisense suppression need not contain in its entirety the AGL1 or AGL5 sequence to be suppressed. Thus, a sense or antisense nucleic acid molecule encoding only a portion of *Arabidopsis* AGL1 (SEQ ID NO:5), for example, or a sense or antisense nucleic acid molecule encoding only a portion of *Arabidopsis* AGL5 (SEQ ID NO:7) can be useful for producing a non-naturally occurring seed plant of the invention, in which AGL1 and AGL5 expression each are suppressed.

A portion of a nucleic acid molecule to be homologously recombined with an AGL1 or AGL5 locus

- generally contains at least about 1 kb of sequence homologous to the targeted gene and preferably contains at least about 2 kb, more preferably at least about 3 kb and can contain at least about 5 kb of sequence
- 5 homologous to the targeted gene. A portion of a nucleic acid molecule encoding an AGL1 or AGL5 to be used for cosuppression or antisense suppression generally contains at least about 50 base pairs to the full-length of the nucleic acid molecule encoding the AGL1 or AGL5 ortholog.
- 10 In contrast to an active segment, as defined herein, a portion of a nucleic acid molecule to be used for homologous recombination, cosuppression or antisense suppression need not encode a functional part of a gene product.
- 15 A dominant negative construct also can be used to suppress AGL1 or AGL5 expression in a seed plant. A dominant negative construct useful in the invention generally contains a portion of the complete AGL1 or AGL5 coding sequence sufficient, for example, for DNA-binding
- 20 or for a protein-protein interaction such as a homodimeric or heterodimeric protein-protein interaction but lacking the transcriptional activity of the wild type protein. For example, a carboxy-terminal deletion mutant of AGAMOUS was used as a dominant negative construct to
- 25 suppress expression of the MADS box gene AGAMOUS (Mizukami et al., Plant Cell 8:831-844 (1996), which is incorporated by reference herein). One skilled in the art understands that, similarly, a dominant negative AGL1 or AGL5 construct can be used to suppress AGL1 or AGL5
- 30 expression in a seed plant. A useful dominant negative construct can be a deletion mutant encoding, for example, the MADS box domain alone ("M"), the MADS box domain and "intervening" region ("MI"); the MADS box, "intervening"

and "K" domains ("MIK"); or the "intervening," "K" and carboxy-terminal domains ("IKC").

In a preferred embodiment, a non-naturally occurring seed plant of the invention is an *agl1 agl5* double mutant. An *agl1 agl5* double mutant is a particularly useful non-naturally occurring seed plant that is characterized by delayed seed dispersal.

As used herein, the term "*agl1 agl5* double mutant" means a seed plant having a loss-of-function mutation at the *AGL1* locus and a loss-of-function mutation at the *AGL5* locus. Loss-of-function mutations encompass point mutations, including substitutions, deletions and insertions, as well as gross modifications of an *AGL1* and *AGL5* locus and can be located in coding or non-coding sequences. One skilled in the art understands that any such loss-of-function mutation at the *AGL1* locus can be combined with any such mutation at the *AGL5* locus to generate an *agl1 agl5* double mutant of the invention. Production of an exemplary *agl1 agl5* double mutant in the Brassica seed plant *Arabidopsis* is disclosed herein in Example II.

*AGL1* and *AGL5* are closely related genes that have diverged relatively recently. While not wishing to be bound by the following, some plants can contain only *AGL1* or only *AGL5*, or can contain a single ancestral gene related to *AGL1* and *AGL5*. In such plants, a seed plant characterized by delayed seed dispersal can be produced by suppressing only expression of *AGL1*, or expression of *AGL5*, or expression of a single ancestral gene related to *AGL1* and *AGL5*. Thus, the present invention provides a non-naturally occurring seed plant characterized by

delayed seed dispersal, in which AGL1 expression is suppressed. Such a non-naturally occurring seed plant characterized by delayed seed dispersal can be, for example, an *agl1* single mutant. The present invention  
5 also provides a non-naturally occurring seed plant characterized by delayed seed dispersal, in which AGL5 expression is suppressed. A non-naturally occurring seed plant characterized by delayed seed dispersal in which AGL5 expression is suppressed can be, for example, an  
10 *agl5* single mutant.

The present invention further provides tissues derived from non-naturally occurring seed plants of the invention. In one embodiment, the invention provides a tissue derived from a non-naturally occurring seed plant  
15 that has an ectopically expressed nucleic acid molecule encoding an AGL8-like gene product and is characterized by delayed seed dispersal. In another embodiment, the invention provides a tissue derived from a non-naturally occurring seed plant in which AGL1 expression and AGL5  
20 expression each are suppressed, where the seed plant is characterized by delayed seed dispersal.

As used herein, the term "tissue" means an aggregate of seed plant cells and intercellular material organized into a structural and functional unit. A  
25 particular useful tissue of the invention is a tissue that can be vegetatively or non-vegetatively propagated such that the seed plant from which the tissue was derived is reproduced. A tissue of the invention can be, for example, a seed, leaf, root or part thereof.

30 As used herein, the term "seed" means a structure formed by the maturation of the ovule of a seed plant following fertilization. Such seeds can be readily

harvested from a non-naturally occurring seed plant of the invention characterized by delayed seed dispersal.

A seed plant characterized by enhanced seed dispersal also can be produced by manipulating expression  
5 of an AGL8-like gene product or AGL1 or AGL5. Suppression of AGL8-like gene product expression in a seed plant, for example, suppression of AGL8-like gene product expression in valve tissue, can be used to  
10 produce a seed plant characterized by enhanced seed dispersal. Ectopic expression of AGL1 or AGL5, or both, in a seed plant, for example, premature expression of AGL1 or AGL5, also can be used to produce a non-naturally occurring seed plant of the invention characterized by enhanced seed dispersal. The skilled person understands  
15 that these or other strategies of manipulating AGL8, AGL1 or AGL5 expression can be used to produce a non-naturally occurring seed plant characterized by enhanced seed dispersal.

The invention also provides a substantially  
20 purified dehiscence zone-selective regulatory element, which includes a nucleotide sequence that confers selective expression upon an operatively linked nucleic acid molecule in the valve margin or dehiscence zone of a seed plant, provided that the dehiscence zone-selective  
25 regulatory element does not have a nucleotide sequence consisting of nucleotides 1889 to 2703 of SEQ ID NO:4.

As used herein, the term "dehiscence  
zone-selective regulatory element" refers to a nucleotide  
sequence that, when operatively linked to a nucleic acid  
30 molecule, confers selective expression upon the operatively linked nucleic acid molecule in a limited number of plant tissues, including the valve margin or



dehiscence zone. As discussed above, the valve margin is the future site of the dehiscence zone and encompasses the margins of the outer replum as well as valve cells adjacent to the outer replum. The dehiscence zone, which  
5 develops in the region of the valve margin, refers to the group of cells that separate during the process of dehiscence, allowing valves to come apart from the replum and the enclosed seeds to be released. Thus, a dehiscence zone-selective regulatory element, as defined  
10 herein, confers selective expression in the mature dehiscence zone, or confers selective expression in the valve margin, which marks the future site of the dehiscence zone.

A dehiscence zone-selective regulatory element  
15 can confer specific expression exclusively in cells of the valve margin or dehiscence zone or can confer selective expression in a limited number of plant cell types including cells of the valve margin or dehiscence zone. An AGL5 regulatory element, for example, which  
20 confers selective expression in ovules and placenta as well as in the dehiscence zone, is a dehiscence zone-selective regulatory element as defined herein. A dehiscence zone-selective regulatory element generally is distinguished from other regulatory elements by  
25 conferring selective expression in the valve margin or dehiscence zone without conferring expression throughout the adjacent carpel valves.

The *Arabidopsis* AGL1 gene (SEQ ID NO:3) is shown in Figure 7, with the intron-exon boundaries  
30 indicated. The *Arabidopsis* AGL5 gene (SEQ ID NO:4) is shown in Figure 8, with the intron-exon boundaries indicated. An AGL1 or AGL5 regulatory element, such as a 5' regulatory element or intronic regulatory element, can confer selective expression in the valve margin or

dehiscence zone and, thus, is a dehiscence-zone selective regulatory element as defined herein. The *AGL5* gene, for example, is selectively expressed in the dehiscence zone, placenta and ovules, and an *AGL5* regulatory element can confer selective expression in the dehiscence zone, placenta and ovules upon an operatively linked nucleic acid molecule.

The invention provides a dehiscence zone-selective regulatory element that is an *AGL1* or *AGL5* regulatory element. Such a dehiscence zone-selective regulatory element can be, for example, an *AGL1* regulatory element. An *AGL1* regulatory element can have, for example, the nucleotide sequence of a non-coding portion of the *Arabidopsis AGL1* genomic sequence identified as SEQ ID NO:3. A dehiscence zone-selective regulatory element also can be, for example, an *AGL5* regulatory element. An *AGL5* regulatory element can have, for example, the nucleotide sequence of a non-coding portion of the *Arabidopsis AGL5* genomic sequence identified as SEQ ID NO:4, provided that the regulatory element does not have a nucleotide sequence consisting of nucleotides 1889 to 2703 of SEQ ID NO:4.

As used herein, the term "substantially the nucleotide sequence," when used in reference to an *AGL1* or *AGL5* regulatory element, means a nucleotide sequence having an identical sequence, or a nucleotide sequence having a similar, non-identical sequence that is considered to be a functionally equivalent sequence by those skilled in the art. For example, a dehiscence zone-selective regulatory element that is an *AGL1* regulatory element can have, for example, a nucleotide sequence identical to the sequence of the *Arabidopsis*

AGL1 regulatory element having nucleotides 1 to 2599 of SEQ ID NO:3 shown in Figure 7, or a similar, non-identical sequence that is functionally equivalent. A dehiscence zone-selective regulatory element can have, for example, one or more modifications such as nucleotide additions, deletions or substitutions relative to the nucleotide sequence shown in Figure 8, provided that the modified nucleotide sequence retains substantially the ability to confer selective expression in the valve margin or dehiscence zone upon an operatively linked nucleic acid molecule.

It is understood that limited modifications can be made without destroying the biological function of an AGL1 or AGL5 regulatory element and that such limited modifications can result in dehiscence zone-selective regulatory elements that have substantially equivalent or enhanced function as compared to a wild type AGL1 or AGL5 regulatory element. These modifications can be deliberate, as through site-directed mutagenesis, or can be accidental such as through mutation in hosts harboring the regulatory element. All such modified nucleotide sequences are included in the definition of a dehiscence zone-selective regulatory element as long as the ability to confer selective expression in the valve margin or dehiscence zone is substantially retained.

A dehiscence zone-selective regulatory element can be derived from a gene that is an ortholog of *Arabidopsis* AGL1 or AGL5 and is selectively expressed in the valve margin or dehiscence zone of a seed plant. A dehiscence zone-selective regulatory element can be derived, for example, from an AGL1 or AGL5 ortholog of the *Brassicaceae*, such as a *Brassica napus*, *Brassica oleracea*, *Brassica campestris*, *Brassica juncea*, *Brassica*

*nigra* or *Brassica carinata* AGL1 or AGL5 ortholog. A dehiscence zone-selective regulatory element can be derived, for example, from an AGL1 or AGL5 canola ortholog. A dehiscence zone-selective regulatory element  
5 also can be derived, for example, from a leguminous AGL1 or AGL5 ortholog, such as a soybean, pea, chickpea, moth bean, broad bean, kidney bean, lima bean, lentil, cowpea, dry bean, peanut, alfalfa, lucerne, birdsfoot trefoil, clover, *stylosanthes*, *lotononis bainesii*, or sainfoin  
10 AGL1 or AGL5 ortholog.

Dehiscence zone-selective regulatory elements also can be derived from a variety of other genes that are selectively expressed in the valve margin or dehiscence zone of a seed plant. For example, the  
15 rapeseed gene RDPG1 is selectively expressed in the dehiscence zone (Petersen et al., Plant Mol. Biol. 31:517-527 (1996), which is incorporated herein by reference). Thus, the RDPG1 promoter or an active fragment thereof can be a dehiscence zone-selective  
20 regulatory element as defined herein. Additional genes such as the rapeseed gene SAC51 also are known to be selectively expressed in the dehiscence zone; the SAC51 promoter or an active fragment thereof also can be a dehiscence zone-selective regulatory element of the  
25 invention (Coupe et al., Plant Mol. Biol. 23:1223-1232 (1993), which is incorporated herein by reference). Further, genes selectively expressed in the dehiscence zone include the gene that confers selective GUS expression in the *Arabidopsis* transposant line GT140  
30 (Sundaresan et al., Genes Devel. 9:1797-1810 (1995), which is incorporated herein by reference). The skilled artisan understands that a regulatory element of any such gene selectively expressed in cells of the valve margin

or dehiscence zone can be a dehiscence zone-selective regulatory element as defined herein.

Additional dehiscence zone-selective regulatory elements can be identified and isolated using routine methodology. Differential screening strategies using, for example, RNA prepared from the dehiscence zone and RNA prepared from adjacent pod material can be used to isolate cDNAs selectively expressed in cells of the dehiscence zone (Coupe et al., *supra*, 1993); subsequently, the corresponding genes are isolated using the cDNA sequence as a probe.

Enhancer trap or gene trap strategies also can be used to identify and isolate a dehiscence zone-selective regulatory element of the invention (Sundaresan et al., *supra*, 1995; Koncz et al., Proc. Natl. Acad. Sci. USA 86:8467-8471 (1989); Kertbundit et al., Proc. Natl. Acad. Sci. USA 88:5212-5216 (1991); Topping et al., Development 112:1009-1019 (1991), each of which is incorporated herein by reference). Enhancer trap elements include a reporter gene such as GUS with a weak or minimal promoter, while gene trap elements lack a promoter sequence, relying on transcription from a flanking chromosomal gene for reporter gene expression. Transposable elements included in the constructs mediate fusions to endogenous loci; constructs selectively expressed in the valve margin or dehiscence zone are identified by their pattern of expression. With the inserted element as a tag, the flanking dehiscence zone-selective regulatory element is cloned using, for example, inverse polymerase chain reaction methodology (see, for example, Aarts et al., Nature 363:715-717 (1993); see, also, Ochman et al., "Amplification of Flanking Sequences by Inverse PCR," in Innis et al.,

*supra*, 1990). The Ac/Ds transposition system of Sundaresan et al., *supra*, 1995, can be particularly useful in identifying and isolating a dehiscence zone-selective regulatory element of the invention.

5 Dehiscence zone-selective regulatory elements also can be isolated by inserting a library of random genomic DNA fragments in front of a promoterless reporter gene and screening transgenic seed plants transformed with the library for dehiscence zone-selective reporter  
10 gene expression. The promoterless vector pROA97, which contains the *npt* gene and the GUS gene each under the control of the minimal 35S promoter, can be useful for such screening. The genomic library can be, for example, Sau3A fragments of *Arabidopsis thaliana* genomic DNA or  
15 genomic DNA from, for example, another *Brassicaceae* of interest (Ott et al., Mol. Gen. Genet. 223:169-179 (1990); Claes et al., The Plant Journal 1:15-26 (1991), each of which is incorporated herein by reference).

Dehiscence zone-selective expression of a  
20 regulatory element of the invention can be demonstrated or confirmed by routine techniques, for example, using a reporter gene and *in situ* expression analysis. The GUS and firefly luciferase reporters are particularly useful for *in situ* localization of plant gene expression  
25 (Jefferson et al., EMBO J. 6:3901 (1987); Ow et al., Science 334:856 (1986), each of which is incorporated herein by reference), and promoterless vectors containing the GUS expression cassette are commercially available, for example, from Clontech (Palo Alto, CA). To identify  
30 a dehiscence zone-selective regulatory element of interest such as an *AGL1* or *AGL5* regulatory element, one or more nucleotide portions of the *AGL1* or *AGL5* gene can be generated using enzymatic or PCR-based methodology



(Glick and Thompson, *supra*, 1993; Innis et al., *supra*, 1990); the resulting segments are fused to a reporter gene such as GUS and analyzed as described above.

The present invention also provides a  
5 substantially purified dehiscence zone-selective  
regulatory element that confers selective expression upon  
an operatively linked nucleic acid molecule in the valve  
margin or dehiscence zone of a seed plant, where the  
element is an *AGL1* regulatory element having at least  
10 fifteen contiguous nucleotides of one of the following  
nucleotide sequences: nucleotides 1 to 2599 of SEQ ID  
NO:3; nucleotides 2833 to 4128 of SEQ ID NO:3;  
nucleotides 4211 to 4363 of SEQ ID NO:3; nucleotides 4426  
to 4554 of SEQ ID NO:3; nucleotides 4655 to 4753;  
15 nucleotides 4796 to 4878 of SEQ ID NO:3; nucleotides 4921  
to 5028 of SEQ ID NO:3; or nucleotides 5361 to 5622 of  
SEQ ID NO:3. A substantially purified dehiscence  
zone-selective regulatory element that is an *AGL1*  
regulatory element can have, for example, at least 16,  
20 18, 20, 25, 30, 40, 50, 100 or 500 contiguous nucleotides  
of one of the portions of SEQ ID NO:3 described above.

The present invention also provides a  
substantially purified dehiscence zone-selective  
regulatory element that confers selective expression upon  
25 an operatively linked nucleic acid molecule in the valve  
margin or dehiscence zone of a seed plant, where the  
element is an *AGL5* regulatory element having at least  
fifteen contiguous nucleotides of one of the following  
nucleotide sequences: nucleotides 1 to 1888 of SEQ ID  
30 NO:4; nucleotides 2928 to 5002 of SEQ ID NO:4;  
nucleotides 5085 to 5204 of SEQ ID NO:4; nucleotides 5367  
to 5453 of SEQ ID NO:4; nucleotides 5496 to 5602;  
nucleotides 5645 to 5734 of SEQ ID NO:4; or nucleotides

6062 to 6138 of SEQ ID NO:4. A substantially purified dehiscence zone-selective regulatory element that is an AGL5 regulatory element can have, for example, at least 16, 18, 20, 25, 30, 40, 50, 100 or 500 contiguous  
5 nucleotides of one of the portions of SEQ ID NO:4 described above.

A proximal fragment of the *Arabidopsis* AGL5 promoter has been described (Savidge et al., The Plant Cell 7:721-733 (1995)). However, this fragment (shown as  
10 nucleotides 1889 to 2703 in Figure 8) lacks many of the distal regulatory elements contained in the entire *Arabidopsis* AGL5 genomic sequence disclosed herein (SEQ ID NO:4). The present invention provides approximately 2.7 kb of *Arabidopsis* AGL5 5' flanking sequence,  
15 including the variety of regulatory elements contained therein. The disclosed *Arabidopsis* AGL5 5' flanking sequence contains a larger complement of regulatory elements involved in regulating expression of the endogenous AGL5 gene *in vivo* and, therefore, can be  
20 particularly useful for dehiscence zone-selective expression.

A nucleotide sequence consisting of the promoter proximal region of *Arabidopsis* AGL5 (nucleotides 1889 to 2703 of SEQ ID NO:4) is explicitly excluded from  
25 a dehiscence zone-selective regulatory element of the invention. However, a dehiscence zone-selective regulatory element can include nucleotides 1889 to 2703 of SEQ ID NO:4, together with one or more contiguous nucleotides, for example, of the nucleotide sequence  
30 shown as positions 1 to 1888 of SEQ ID NO:4. A dehiscence zone-selective regulatory element of the invention can have, for example, at least 15 contiguous nucleotides of SEQ ID NO:4, including at least one, two,

four, six, ten, twenty or thirty or more contiguous nucleotides of the nucleotide sequence shown as positions 1 to 1888 of SEQ ID NO:4.

5           In view of the definition of a dehiscence zone-selective regulatory element, it should be recognized, for example, that a portion of the *Arabidopsis* AGL5 gene having only the sequence shown as nucleotides 1889 to 2703 in Figure 8 (SEQ ID NO:4), is  
10 not a dehiscence zone-selective regulatory element as defined herein. However, a portion of an *Arabidopsis* AGL5 gene having nucleotides 1885 to 2703 of SEQ ID NO:4 is considered a dehiscence zone-selective regulatory  
15 element, provided that the element confers selective expression upon an operatively linked nucleic acid molecule in a limited number of plant tissues, including the valve margin or dehiscence zone. Similarly, a portion of an *Arabidopsis* AGL5 gene having a subpart of the promoter proximal region of AGL5 also can be a  
20 dehiscence zone-selective regulatory element as defined herein, provided that this subpart can confer selective expression upon an operatively linked nucleic acid molecule in a limited number of plant tissues, including the valve margin or dehiscence zone of a seed plant.  
25 Thus, for example, a regulatory element having the sequence of nucleotides 1889 to 2000 can be a dehiscence zone-selective regulatory element of the invention, provided that this element confers selective expression upon an operatively linked element in the valve margin or  
30 dehiscence zone of a seed plant.

The present invention also provides a recombinant nucleic acid molecule that includes a dehiscence zone-selective regulatory element operatively linked to a nucleic acid molecule encoding a cytotoxic

gene product. Further provided herein is a non-naturally occurring seed plant of the invention that is characterized by delayed seed dispersal due to expression of a recombinant nucleic acid molecule having a  
5 dehiscence zone-selective regulatory element operatively linked to a nucleic acid molecule encoding a cytotoxic gene product.

A cytotoxic gene product is a gene product that causes the death of the cell in which it is expressed  
10 and, preferably, does not result in the death of cells other than the cell in which it is expressed. Thus, expression of a cytotoxic gene product from a dehiscence zone-selective regulatory element can be used to ablate the dehiscence zone without disturbing neighboring cells  
15 of the replum or valve. A variety of cytotoxic gene products useful in seed plants are known in the art including, for example, diphtheria toxin A chain polypeptides; RNase T1; Barnase RNase; ricin toxin A chain polypeptides; and herpes simplex virus thymidine  
20 kinase (tk) gene products. While the diphtheria toxin A chain, RNase T1 and Barnase RNase are preferred cytotoxic gene products, the skilled person recognizes that these, or other cytotoxic gene products can be used with a dehiscence zone-selective regulatory element to generate  
25 a non-naturally occurring seed plant characterized by delayed seed dispersal.

Diphtheria toxin is the naturally occurring toxin of *Cornebacterium diphtheriae*, which catalyzes the ADP-ribosylation of elongation factor 2, resulting in  
30 inhibition of protein synthesis and consequent cell death (Collier, Bacteriol. Rev. 39:54-85 (1975)). A single molecule of the fully active toxin is sufficient to kill a cell (Yamaizumi et al., Cell 15:245-250 (1978)).

Diphtheria toxin has two subunits: the diphtheria toxin B chain directs internalization to most eukaryotic cells through a specific membrane receptor, whereas the A chain encodes the toxic catalytic domain. The catalytic DT-A chain does not include a signal peptide and is not secreted. Further, any DT-A released from dead cells in the absence of the diphtheria toxin B chain is precluded from cell attachment. Thus, DT-A is cell autonomous and directs killing only of the cells in which it is expressed without apparent damage to neighboring cells. The DT-A expression cassette of Palmiter et al., which contains the 193 residues of the A chain engineered with a synthetic ATG and lacking the native leader sequence, is particularly useful in the seed plants of the invention (Palmiter et al., Cell 50:435-443 (1987); Greenfield et al., Proc. Natl. Acad. Sci., USA 80:6853-6857 (1983), each of which is incorporated herein by reference).

20 RNase T1 of *Aspergillus oryzae* and Barnase  
gene products useful in the seed plants of the invention  
(Thorsness and Nasrallah, Methods in Cell Biology  
50:439-448 (1995)). Barnase RNase may be more generally  
25 toxic to plants than RNase T1 and, thus, is preferred in  
the methods of the invention.

Ricin, a ribosome-inactivating protein produced  
by castor bean seeds, also is a cytotoxic gene product  
useful in a non-naturally occurring seed plant of the  
invention. The ricin toxin A chain polypeptide can be  
30 used to direct cell-specific ablation as described, for  
example, in Moffat et al., Development 114:681-687  
(1992). Plant ribosomes are variably susceptible to the  
plant-derived ricin toxin. The skilled person

understands that the toxicity of ricin depends is variable and should be assessed for toxicity in the seed plant species of interest (see Olsnes and Pihl, Molecular Action of Toxins and Viruses, pages 51-105, Amsterdam: Elsevier Biomedical Press (1982)).

Further provided herein is a plant expression vector including a dehiscence zone-selective regulatory element. A plant expression vector can include, if desired, a nucleic acid molecule encoding an AGL8-like gene product in addition to the dehiscence zone-selective regulatory element.

The term "plant expression vector," as used herein, is a self-replicating nucleic acid molecule that provides a means to transfer an exogenous nucleic acid molecule into a seed plant host cell and to express the molecule therein. Plant expression vectors encompass vectors suitable for *Agrobacterium*-mediated transformation, including binary and cointegrating vectors, as well as vectors for physical transformation.

Plant expression vectors can be used for transient expression of the exogenous nucleic acid molecule, or can integrate and stably express the exogenous sequence. One skilled in the art understands that a plant expression vector can contain all the functions needed for transfer and expression of an exogenous nucleic acid molecule; alternatively, one or more functions can be supplied in trans as in a binary vector system for *Agrobacterium*-mediated transformation.

In addition to a dehiscence zone-selective regulatory element, a plant expression vector of the invention can contain, if desired, additional elements.



A binary vector for *Agrobacterium*-mediated transformation contains one or both T-DNA border repeats and can also contain, for example, one or more of the following: a broad host range replicon, an *ori T* for efficient transfer from *E. coli* to *Agrobacterium*, a bacterial selectable marker such as ampicillin and a polylinker containing multiple cloning sites.

A plant expression vector for physical transformation can have, if desired, a plant selectable marker in addition to a dehiscence zone-selective regulatory element in vectors such as pBR322, pUC, pGEM and M13, which are commercially available, for example, from Pharmacia (Piscataway, NJ) or Promega (Madison, WI). In plant expression vectors for physical transformation of a seed plant, the T-DNA borders or the *ori T* region can optionally be included but provide no advantage.

The present invention also provides a kit for producing a transgenic seed plant characterized by delayed seed dispersal. A kit of the invention contains a dehiscence zone-selective regulatory element. If desired, the dehiscence zone-selective regulatory element can be operatively linked to a nucleic acid molecule encoding an AGL8-like gene product.

The following examples are intended to illustrate but not limit the present invention.

## EXAMPLE I

PRODUCTION OF A 35S-AGL8 TRANSGENIC ARABIDOPSIS PLANT  
DISPLAYING A COMPLETE LACK OF DEHISCENCE

This example describes methods for producing a  
5 transgenic *Arabidopsis* plant lacking normal dehiscence  
due to constitutive AGL8 expression.

Full-length AGL8 was prepared by polymerase  
chain reaction amplification using primer AGL8 5-γ (SEQ  
ID NO:9; 5'-CCGTCGACGATGGGAAGAGGTAGGGTT-3') and primer  
10 OAM14 (SEQ ID NO:10; 5'-AATCATTACCAAGATATGAA-3'), and  
subsequently cloned into the SalI and BamHI sites of  
expression vector pBIN-JIT, which was modified from  
pBIN19 to include the tandem CaMV 35S promoter, a  
polycloning site and the CaMV polyA signal. *Arabidopsis*  
15 was transformed using the *in planta* method of  
*Agrobacterium*-mediated transformation essentially as  
described in Bechtold et al., C.R. Acad. Sci. Paris  
316:1194-1199 (1993), which is incorporated herein by  
reference. Kanamycin-resistant lines were analyzed for  
20 the presence of the 35S-AGL8 construct by PCR using a  
primer specific for the 35S promoter and a primer  
specific for the AGL8 cDNA, which produced two fragments  
of 850 and 550 bp in the 35S-AGL8 transgenic plants.  
These fragments were absent in plants that had not been  
25 transformed with the 35S-AGL8 construct.

The phenotype of approximately 35 35S::AGL8  
lines was analyzed. Of the 35 lines, 7 lines exhibited a  
complete lack of dehiscence. In these lines, the mature  
fruits did not release their seeds unless opened  
30 manually. Several of the remaining 35S::AGL8 lines  
exhibited delayed dehiscence, whereby seeds were released  
at least a week later than in wild type *Arabidopsis*  
plants.

**EXAMPLE II**

PRODUCTION OF AN ARABIDOPSIS *agl1 agl5* double mutant  
DISPLAYING A COMPLETE LACK OF DEHISCENCE

This example describes the production of an  
5 *agl1 agl5* double mutant displaying a complete lack of  
normal dehiscence.

A. Production of an *agl5* mutant by homologous  
recombination

A PCR-based assay of transgenic plants was used  
10 to identify targeted insertions into *AGL5* as described in  
Kempin et al., Nature 389:802-803 (1997), which is  
incorporated herein by reference. The targeting  
construct consisted of a kanamycin-resistance cassette  
that was inserted between approximately 3 kb  
15 and 2 kb segments representing the 5' and 3' regions of  
the *AGL5* gene, respectively. A successfully targeted  
insertion produces a 1.6 kb deletion within the *AGL5* gene  
such that the targeted allele encodes only the first 42  
of 246 amino acid residues, and only 26 of the 56 amino  
20 acids comprising the DNA-binding MADS-domain. The  
recombination event also results in the insertion of the  
2.5 kb kanamycin-resistance cassette within the *AGL5*  
coding sequence.

750 kanamycin-resistant transgenic lines were  
25 produced by *Agrobacterium*-mediated transformation, and  
pools of transformants were analyzed using a PCR assay as  
described below to determine if any of these primary  
transformants had generated the desired targeted  
insertion into *AGL5*. A single line was identified that  
30 appeared to contain the anticipated insertion, and this  
line was allowed to self-pollinate to permit further

analyses in subsequent generations. Genomic DNA from the homozygous mutant plants was analyzed with more than four different restriction enzymes and by several distinct PCR amplifications, and all data were consistent with the  
5 desired targeting event. The regions flanking the *AGL5* gene also were analyzed to verify that there were no detectable deletions or rearrangements of sequences outside of *AGL5*.

The kanamycin-resistance cassette within the  
10 *AGL5* targeting construct contains sequences that specify transcription termination such that little or no *AGL5* RNA was expected in the homozygous mutant plants. Using a probe specific for the 3' portion of the *AGL5* cDNA, *AGL5* transcripts were detected in wild-type but not in *agl5*  
15 mutant plants. These data indicate that the targeted disruption of the *AGL5* gene represents a loss-of-function allele.

Characterization of the *agl5* line indicated that the phenotype of this transgenic was not different  
20 from wild type *Arabidopsis*.

The *AGL5* knockout (KO) construct was prepared in vector pZM104A, which carries the kanamycin-resistance cassette flanked by several cloning sites (Miao and Lam, Plant J. 7:359-365 (1995), which is incorporated herein  
25 by reference). Vector pZM104A also contains the gene encoding  $\beta$ -glucuronidase (GUS), which allows the differentiation of non-homologous from homologous integration events. The 3 kb region representing the 5' portion of *AGL5* was obtained by PCR amplification using  
30 primer SEQ ID NO:11 (5'-CGGATAGCTCGAATATCG-3') and primer SEQ ID NO:12 (5'-AACCATTGCGTCGTTTGC-3'). The resulting fragment was cloned into vector pCRII (Invitrogen), and

an EcoRI fragment excised and inserted into the EcoRI site of pZM104A. The 3' portion of *AGL5* was excised as an XbaI fragment from an *AGL5* genomic clone in the vector pCIT30 (Ma et al., Gene 117:161-167 (1992), which is  
5 incorporated by reference herein) and inserted into the XbaI site of pZM104A. The resulting plasmid, designated *AGL5* KO, was used in *Agrobacterium*-mediated infiltration of wild-type *Arabidopsis* plants of the Columbia ecotype. The knockout construct was derived from Landsberg erecta  
10 genomic DNA.

Plants containing a homologous recombination event at the *AGL5* genomic locus were identified as follows. Approximately 750 primary (T1) kanamycin-resistant transformants were selected, and DNA  
15 was extracted from individual leaves in pools representing ten plants as described in Edwards et al., Nucleic Acids Research 19:1349 (1991), which is incorporated by reference herein. To identify a pool that contained a candidate targeted disruption, isolated  
20 DNAs were subjected to PCR amplification using primer SEQ ID NO:13 (5'-GTAATTACCAGGCAAGGACTCTCC-3'), which represents *AGL5* genomic sequence that is not contained within the *AGL5* KO construct, and primer SEQ ID NO:14 (5'-GTCATCGGCGGGGGTCATAACGTG-3'), which is specific for  
25 the kanamycin-resistance cassette. Amplified products were size fractionated on agarose gels, and used for standard DNA blotting assays with probe 1. One pool of ten plants revealed the anticipated hybridizing band of the correct size, and this pool was subsequently  
30 broken down into individual plants. A single (T1) plant was identified that appeared to contain the desired event, and this plant was allowed to self-pollinate for analyses in subsequent generations.

This T1 plant was shown to contain the GUS-reporter gene, indicating that in addition to the putative homologous integration event, there were independent non-homologous events. Segregation in the subsequent generations allowed the identification of plants that no longer contained the GUS-reporter gene, and it was these lines that were used for subsequent analyses.

Plants homozygous for the disruption were identified by PCR amplification using primers SEQ ID NO:15 (5'-GAGGATAGAGAACACTACGAATCG-3') and SEQ ID NO:16 (5'-CAGGTCAAGTCAATAGATTTC-3'), which yielded a single 1.5 kb product in wild type plants, and a single 2.6 kb product in the mutant. Further confirmation that these plants contained the desired disruption was obtained by PCR amplification with primers SEQ ID NO:17 (5'-CAGAATTTAGTGAATAATATTG-3') and SEQ ID NO:14, which gave the expected amplified product in the mutant but no product in wild-type plants.

To confirm that the desired disruption had occurred, a series of genomic DNA blots representing wild-type and homozygous mutant (T4 generation) plants were analyzed. Probe 1 hybridized to the expected 3.9 kb XbaI fragment in wild-type and mutant plants, whereas the 1.3 kb XbaI fragment was present only in wild-type. This same probe hybridized to a 6 kb EcoRI fragment in wild-type and to the expected 4.1 and 2.8 kb EcoRI fragments in the mutant. Additional digests with BglIII and with HindIII confirmed that the mutant plants contained the desired targeted event. To confirm that there were no detectable deletions or rearrangements outside the targeted region, genomic DNA blots of wild type and homozygous mutant plants were further analyzed. Probe 2 hybridized in wild-type and mutant DNAs to the



expected 2.9 kb XmnI fragment, the 1.5 kb and 0.4 kb HincII fragments, and the 0.6 kb HindIII fragment. Probe 3 hybridized in wild-type and mutant DNAs to the 9 kb ScaI fragment, the 3.9 kb XbaI fragment, and the 1.8 kb NdeI fragments. The faintly-hybridizing bands in the ScaI digests represent fragments that span the insertion site, and are, as expected, different sizes in wild-type and *agl5* mutant plants.

RNA blotting analyses were performed as follows. Approximately 6  $\mu$ g of polyA<sup>+</sup> RNA was purified using Dynabeads (Dynal) from wild-type and *agl5* mutant inflorescences, size fractionated and hybridized using standard procedures (Crawford et al., Proc. Natl. Acad. Sci. USA 83:8073-8076 (1986), which is incorporated herein by reference) using a gel-purified 450 bp HindIII-EcoRI fragment from pCIT2242 (Ma et al., *supra*, 1991) specific for the 3' end of the *AGL5* cDNA. The same filter was subsequently stripped and re-hybridized with a tubulin-specific probe (Marks et al., Plant Mol. Biol. 10:91-104 (1987), which is incorporated herein by reference). Hybridization with the tubulin probe verified that approximately equal amounts of RNA were present in each lane.

#### B. Production of an *agl1* mutant

A PCR-based screen was used to identify a T-DNA insertion into the *AGL1* gene essentially as described in Krysan et al., *supra*, 1996.

RNA blotting analyses demonstrated that *AGL1* RNA was not expressed. The *agl1* mutant displayed essentially a wild type phenotype.

C. Production and characterization of an *agl1 agl5* double mutant

*agl1 agl5* double mutants were generated by crossing the *agl1* and *agl5* single mutants. RNA blotting experiments of the *agl1 agl5* double mutant are performed as described above. The results indicate that neither *AGL1* nor *AGL5* RNA is expressed in the *agl1 agl5* double mutant.

In contrast to the *agl1* and *agl5* single mutants, which had essentially the phenotype of wild type *Arabidopsis*, analyses of the *agl1 agl5* double mutant by scanning electron microscopy indicated that the dehiscence zone failed to develop normally. Furthermore, the mature fruits of the *agl1 agl5* double mutant failed to dehisce. This delayed seed dispersal phenotype was similar to *AGL8* gain-of-function phenotype seen in 35S-*AGL8* transgenic plants. These results indicate that the *AGL1* and *AGL5* genes are functionally redundant and that their encoded gene products regulate pod dehiscence. The similarity of the 35S::*AGL8* and *agl1 agl5* double mutant phenotypes, as well the yeast two-hybrid results described below, indicate that *AGL1* and *AGL8* or *AGL5* and *AGL8* can interact to regulate the dehiscence process.

D. Analysis of dehiscence phenotypes under various conditions

Studies of pod dehiscence in *Brassica napus* L. using transmission electron microscopic analyses have shown that the middle lamella of the dehiscence zone cells degenerates during dehiscence, allowing the valves to separate from the replum (Petersen et al.,

*supra*, 1996). Similar analyses are performed on the *agl1* *agl5* double mutant as well as wild type *Arabidopsis* and *agl1* and *agl5* single mutants.

Previous studies have shown that pod dehiscence is greater when temperatures are high and the relative humidity is low. The dehiscence phenotype of the *agl1* *agl5* double mutant described above was observed for plants grown under continuous-light at 25 degrees C. In order to determine if the phenotype of *agl1* *agl5* double mutants is sensitive to environmental conditions, the analyses described above are repeated under various environmental conditions including varying temperature, varying humidity and short-day versus continuous light conditions.

15

### EXAMPLE III

#### PRODUCTION OF A TRANSGENIC ARABIDOPSIS PLANT EXPRESSING AGL8 UNDER CONTROL OF THE AGL1 PROMOTER

This example demonstrates that a transgenic seed plant expressing AGL8 under control of a dehiscence zone-selective promoter is characterized by delayed seed dispersal.

#### AGL1::AGL8 transgenic plants

Ectopic expression of AGL8 under control of the 35S promoter prevents pod shatter since the dehiscence zone fails to differentiate normally. However, constitutive AGL8 expression conferred by the 35S promoter also results in other changes, including early flowering. In order to specifically control dehiscence, AGL8 is expressed from a dehiscence zone-selective regulatory element, such as one derived from a regulated

promoter that is normally expressed in valve margin, as described below.

An AGL8 expression construct under control of the dehiscence zone-selective 2.5 kb AGL1 promoter fragment and first AGL1 intronic sequence is prepared as follows. The 2.5 kb AGL1 promoter fragment is amplified by PCR with primers AGL1pds (SEQ ID NO:18; 5'-GCCAGAGATAATGCTATTCC-3') and AGL1pus (SEQ ID NO:19; 5'-CATTGATCCATATATGACATCAC-3'), and the first coding exon of AGL8 is amplified with oligos AGL8eds (SEQ ID NO:20; 5'-GTGATGTCATATATGGATCAATGGGAAGAGGTAGGGTTCAG-3') and AGL8eus (SEQ ID NO:21; 5'-CAAGAGTCGGTGGAATATTCG-3'). In addition, the first intron of AGL1, which can contain regulatory elements, is amplified with oligos AGL1ids (SEQ ID NO:22; 5'-CGAATATTCCACCGACTCTTGGTACGCTTC TCCTACTCTAT-3') and AGL1iup (SEQ ID NO:23; 5'-CTAATAAGTAAGATCGCGGAA-3'). The remainder of the AGL8 coding region is amplified with oligos AGL8rds (SEQ ID NO:24; 5'-TTCCGCGATCTTACTTATTAGCATGGAGAGGATACTTGAAC-3') and OAM14 (SEQ ID NO:10). Using PCR with oligos AGL1pds (SEQ ID NO:18) and OAM14 (SEQ ID NO:10), the four fragments are combined in the following order: AGL1 promoter, first AGL8 exon, first AGL1 intron and remainder of AGL8 coding sequence. The resulting 4.6 kb fragment is cloned into vector pCFM83, which is a vector based on pBIN19 that is modified to contain a BASTA resistance gene and 3' NOS termination sequence.

A second AGL8 expression construct, in which AGL8 is under control of the dehiscence zone-selective 2.5 kb AGL1 promoter fragment alone, is prepared as follows. The 2.5 kb AGL1 promoter fragment is amplified by PCR with oligo AGL1pds (SEQ ID NO:18) and AGL1pus (SEQ ID NO:19), and the coding region of AGL8 amplified with

oligos AGL8eds (SEQ ID NO:20) and OAM14 (SEQ ID NO:10). Using PCR with oligos AGL1pds (SEQ ID NO:18) and OAM14 (SEQ ID NO:10), the 3.5 kb fragment is cloned into vector pCFM83.

5                    *Arabidopsis* plants are transformed with the two AGL1-AGL8 constructs described above. BASTA resistant plants containing the AGL1::AGL8 transgene with or without the AGL1 intron are selected. Phenotypic analysis indicates that transformed plants containing  
10 either of these constructs are characterized by delayed dehiscence. However, the AGL1::AGL8 transgenic plants differ from 35S::AGL8 transgenic plants in that an enlarged fruit or early flowering phenotype generally is not seen.

15                    These results indicate that a transgenic seed plant expressing AGL8 under control of an *AGL1* dehiscence zone-selective regulatory element is characterized by delayed seed dispersal.

#### EXAMPLE IV

##### 20                    AGL8 INTERACTS WITH AGL5 IN YEAST

This example demonstrates that, in a yeast two-hybrid system, the AGL8 gene product interacts with AGL5.

The "interaction trap" of Finley and Brent  
25                    (Gene Probes: A Practical Approach (1994); see, also Gyuris et al., Cell 75:791-803 (1993)) is a variation of the yeast two-hybrid system of Fields and Song, Nature 340:245-246 (1989). In this system, a first protein is fused to a DNA-binding domain, and a second is fused to a  
30 transcriptional activation domain. An interaction

between the *Arabidopsis* AGL5 and AGL8 gene products was assayed by activation of a *lacZ* reporter gene.

The "bait" and "prey" constructs were prepared in single copy centromere plasmids pBI-880 and pBI-771, respectively, which each contain the constitutive ADHI promoter and are essentially as described by Chevray and Nathans, Proc. Natl. Acad. Sci. USA 89:5789-5793 (1992). The bait construct contains the GAL4 DNA-binding domain (amino acids 1 to 147) fused to the full-length AGL8 coding sequence. The prey construct has the full-length coding sequence of AGL5 fused to the GAL4 transcriptional activation domain (amino acids 768-881), following a nuclear localization sequence. The bait and prey constructs were assayed in the YPB2 strain of *S. cerevisiae*, which is deficient for *GAL4* and *GAL80* and which contains an integrated *lacZ* reporter gene under control of *GAL1* promoter elements (Feilotter et al., Nucleic Acids Research 22:1502-1503 (1994)).

An interaction of the AGL8 "bait" and AGL5 "prey" was demonstrated in the YPB2 strain by the development of blue colonies on X-GAL containing media. Control "bait"- "prey" combinations, including the GAL4(1-147) DNA binding domain and GAL4 transcriptional activation domain only produced only white colonies. These results demonstrate that AGL8 can interact with AGL5 in yeast and indicate that the AGL8 and AGL5 plant MADS box gene products also can interact in seed plants.

All journal article, reference, and patent citations provided above, in parentheses or otherwise, whether previously stated or not, are incorporated herein by reference.



Although the invention has been described with reference to the examples above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the  
5 invention is limited only by the following claims.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: The Regents of the University of California
- (ii) TITLE OF INVENTION: Seed Plants Characterized by Delayed Seed Dispersal
- (iii) NUMBER OF SEQUENCES: 24
- (iv) CORRESPONDENCE ADDRESS:
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  - (D) STATE: California
  - (E) COUNTRY: United States
  - (F) ZIP: 92122
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 60/051,030
  - (B) FILING DATE: 27-JUN-1997
  
  - (A) APPLICATION NUMBER: US 09/067,800
  - (B) FILING DATE: 28-APR-1998
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Campbell, Cathryn A.
  - (B) REGISTRATION NUMBER: 31,815
  - (C) REFERENCE/DOCKET NUMBER: FP-UD 3188
- (ix) TELECOMMUNICATION INFORMATION:
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  - (B) TELEFAX: (619) 535-8949

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1062 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 101..827

## (ix) FEATURE:

(A) NAME/KEY: misc\_feature

(B) LOCATION: 1062

(D) OTHER INFORMATION: /note= "There is a poly(A) tail at the end."

## (ix) FEATURE:

(A) NAME/KEY: misc\_feature

(B) LOCATION: 1..1062

(D) OTHER INFORMATION: /note= "Nucleotide and Deduced Amino Acid Sequences of the AGL8 cDNA clone."

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCCAGAGAGA CATAAGAAAG AAAGAGAGAG AGAGATACTT TGGTCATTTC AGGGTTGTCG	60
TTTCTCTCTC TTGTTCTTGA GATTTTGAAG AGAGAGAGAT ATG GGA AGA GGT AGG	115
Met Gly Arg Gly Arg	5
1	
GTT CAG CTG AAG AGG ATA GAG AAC AAG ATC AAT AGG CAA GTT ACT TTC	163
Val Gln Leu Lys Arg Ile Glu Asn Lys Ile Asn Arg Gln Val Thr Phe	20
10	15
TCA AAG AGA AGG TCT GGT TTG CTC AAG AAA GCT CAT GAG ATC TCT GTT	211
Ser Lys Arg Arg Ser Gly Leu Leu Lys Lys Ala His Glu Ile Ser Val	35
25	30
CTC TGC GAT GCT GAG GTT GCT CTC ATC GTC TTC TCT TCC AAA GGC AAA	259
Leu Cys Asp Ala Glu Val Ala Leu Ile Val Phe Ser Ser Lys Gly Lys	50
40	45
CTC TTC GAA TAT TCC ACC GAC TCT TGC ATG GAG AGG ATA CTT GAA CGC	307
Leu Phe Glu Tyr Ser Thr Asp Ser Cys Met Glu Arg Ile Leu Glu Arg	65
55	60
TAT GAT CGC TAT TTA TAT TCA GAC AAA CAA CTT GTT GGC CGA GAC GTT	355
Tyr Asp Arg Tyr Leu Tyr Ser Asp Lys Gln Leu Val Gly Arg Asp Val	85
70	75
TCA CAA AGT GAA AAT TGG GTT CTA GAA CAT GCT AAG CTC AAG GCA AGA	403
Ser Gln Ser Glu Asn Trp Val Leu Glu His Ala Lys Leu Lys Ala Arg	100
90	95
GTT GAG GTA CTT GAG AAG AAC AAA AGG AAT TTT ATG GGG GAA GAT CTT	451
Val Glu Val Leu Glu Lys Asn Lys Arg Asn Phe Met Gly Glu Asp Leu	115
105	110
GAT TCG TTG AGC TTG AAG GAG CTC CAA AGC TTG GAG CAT CAG CTC GAT	499
Asp Ser Leu Ser Leu Lys Glu Leu Gln Ser Leu Glu His Gln Leu Asp	130
120	125
GCA GCT ATC AAG AGC ATT AGG TCA AGA AAG AAC CAA GCT ATG TTC GAA	547
Ala Ala Ile Lys Ser Ile Arg Ser Arg Lys Asn Gln Ala Met Phe Glu	145
135	140
TCC ATA TCT GCG CTC CAG AAG AAG GAT AAA GCC TTG CAA GAT CAC AAC	595
Ser Ile Ser Ala Leu Gln Lys Lys Asp Lys Ala Leu Gln Asp His Asn	165
150	155
AAT TCG CTT CTC AAA AAG ATT AAG GAG AGG GAG AAG AAA ACG GGT CAG	643
Asn Ser Leu Leu Lys Lys Ile Lys Glu Arg Glu Lys Lys Thr Gly Gln	

78

170	175	180	
CAA GAA GGA CAA TTA GTC CAA TGC	TCC AAC TCT TCT TCA GTT CTT CTG		691
Gln Glu Gly Gln Leu Val Gln Cys	Ser Asn Ser Ser Ser Val Leu Leu		
185	190	195	
CCT CAA TAC TGC GTA ACC TCC TCC	AGA GAT GGC TTT GTG GAG AGA GTT		739
Pro Gln Tyr Cys Val Thr Ser Ser	Arg Asp Gly Phe Val Glu Arg Val		
200	205	210	
GGG GGA GAG AAC GGT GGT GCA TCG	TCG TTG ACG GAA CCA AAC TCT CTG		787
Gly Gly Glu Asn Gly Gly Ala Ser	Ser Leu Thr Glu Pro Asn Ser Leu		
215	220	225	
CTT CCG GCT TGG ATG TTA CGT CCT	ACC ACT ACG AAC GAG T AGAACTATCT		837
Leu Pro Ala Trp Met Leu Arg Pro	Thr Thr Thr Asn Glu		
230	235	240	
CACTCTTTAT AATATAATGA TAATATAATT AATGTTTAAT ATTTTCATAA CATTCAGCAT			897
TTTTTTGGTG ACTTATACTC ATTATTAATA CCGATATGTT TTAGCTAGTC ATATTATATG			957
TATGATGGAA CTCGTTGTC GAGACGTATG TACGTAAGCT ATCATTAGAT TCACTGCGTC			1017
TTAAGAACAA AGATTCATAT CTTGGTAATG ATTTCTCATG AAATA			1062

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 242 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Gly	Arg	Gly	Arg	Val	Gln	Leu	Lys	Arg	Ile	Glu	Asn	Lys	Ile	Asn
1				5					10					15	
Arg	Gln	Val	Thr	Phe	Ser	Lys	Arg	Arg	Ser	Gly	Leu	Leu	Lys	Lys	Ala
		20						25					30		
His	Glu	Ile	Ser	Val	Leu	Cys	Asp	Ala	Glu	Val	Ala	Leu	Ile	Val	Phe
		35					40					45			
Ser	Ser	Lys	Gly	Lys	Leu	Phe	Glu	Tyr	Ser	Thr	Asp	Ser	Cys	Met	Glu
	50					55					60				
Arg	Ile	Leu	Glu	Arg	Tyr	Asp	Arg	Tyr	Leu	Tyr	Ser	Asp	Lys	Gln	Leu
65				70					75					80	
Val	Gly	Arg	Asp	Val	Ser	Gln	Ser	Glu	Asn	Trp	Val	Leu	Glu	His	Ala
			85						90					95	
Lys	Leu	Lys	Ala	Arg	Val	Glu	Val	Leu	Glu	Lys	Asn	Lys	Arg	Asn	Phe
		100						105					110		
Met	Gly	Glu	Asp	Leu	Asp	Ser	Leu	Ser	Leu	Lys	Glu	Leu	Gln	Ser	Leu
		115					120					125			
Glu	His	Gln	Leu	Asp	Ala	Ala	Ile	Lys	Ser	Ile	Arg	Ser	Arg	Lys	Asn

79

130

135

140

Gln	Ala	Met	Phe	Glu	Ser	Ile	Ser	Ala	Leu	Gln	Lys	Lys	Asp	Lys	Ala
145					150					155					160

[illegible]

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5622 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: unknown  
(D) TOPOLOGY: unknown

(ix) FEATURE:

- ```
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..5622
(D) OTHER INFORMATION: /label= AGL1_promoter
                        /note= "Nucleotide sequence of the AGL1 promoter."
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

|            |            |            |            |            |            |     |
|------------|------------|------------|------------|------------|------------|-----|
| AGATCTGCAA | CAGTGAAAAG | AGAAAACAAA | ATGGACTTGA | AGAGGTTTTG | ACAATGCCAG | 60  |
| AGATAATGCT | TATTCCCTAA | TATGTTGCCA | GCCAAGTGTC | AAATTGGCTT | TTTAAATATG | 120 |
| GATTTCTGTA | TCAGTGGTCA | TATTTGTGGA | TCCAACGTAT | TCATCATCAA | GTTCTCAAGT | 180 |
| TTGCTTTCAG | TGCAATTCTA | ATTCACACGT | TTAACTTTAA | CATGCATCTC | ATTATAATTA | 240 |
| CTTCTTCACT | AAGACACAAT | ACGGCAAACC | TTTCAGATTA | TATTAATCTC | CATAAATGAA | 300 |
| ATAATTAACC | TCATAATCAA | GATTCAATGT | TTCTAAATAT | ATATGGACAA | AATTTACACG | 360 |
| GAAGATTAGA | TACGTATATT | AGTAGATTTA | GTCTTTCGTT | TGTGCGATAA | GATTAACCAC | 420 |
| CTCATAGATA | GTAATATCAT | TGTCAAATTC | CTCTCGGTTT | AGTCGCTAAA | TTGTATCTTT | 480 |
| TTTAAGCCTA | AAAGTAGTGT | ATTCGCATAT | GACTTATCGT | CCTAACTTTT | TTTTTAATTA | 540 |
| ACAAAAAAAT | CGAAAAGAAA | ATAATCTGTT | AAATATTTTT | TAAGTACTCC | ATTAAGTTTA | 600 |
| GTTTCTATTT | AAAAAATGCT | TGAAATTTGA | CAGTTATGTT | CAACAATTTT | GAATCATGAG | 660 |
| CGATGTCTAG | ATACTCAGAA | TTTAATCAAG | ATGTCTTATC | AAATTTGTTG | TCACTCGAGG | 720 |
| ACCCACGCAA | AAGAAAAGAC | TAATATGATT | TTTATTTGGT | CTGGATATTT | TTGTAGAGGA | 780 |



|                                                                   |      |
|-------------------------------------------------------------------|------|
| TGAAACTAAG AGAGTGAAAG ATTCGAAATC CACAATGTTC AAGAGAGCTC AAAGCAAAAA | 840  |
| GAAAAATGAA GATGAAGGAC TAAAGAACAA TAAGCAACTA CTTATACCCT ATTTCCATAA | 900  |
| AGGATTCAGG TACTAGGAGA AGTTGAGGCA AGTTNNNNNN NATTGATTCA AATTTTCATT | 960  |
| TATTTTACA ATTTAATTCA CCTAAGTTAT TATGCATTTT TCATCATTGG TACATTTTCT  | 1020 |
| GTATAGCGTA TTTACATATA TGAAATAAAT TAAATATGTC CTCACGTTGC AAGTAGTTAA | 1080 |
| TGAATGTCCC CACGCAAAAA AAAATCCCTC CAAATATGTC CACCTTTTCT TTTCTTTTAA | 1140 |
| ATTCCAAAAT TACCATAAAC TTTTGGTTTA CAAAAGATTT CTAGAAATTG AGGAAGATAT | 1200 |
| CCTAAATGAT TCATGAATCC TTCAATAATC TGAAGTTTGC GATATTTTCG ATTTTCTTCA | 1260 |
| AGAGTTGCGA TATTTGTAAT TTGGTGACCT TAACTTTTTT TTGATAAAGA GTAAACGTTT | 1320 |
| TTTCTTAAAA GTAAACTTGT ATTTTATGTT TTAGGGTTCT AGCTCAACTT TGTATTATAT | 1380 |
| TTCTTGCAAA AAGAGTTCGT TAACTGCATT CTTCAACACT ATAAAGTGAT TATCAAAAAC | 1440 |
| ATCTTCATGA ACATTAAGAA AAACAATATT TGGTTTCGGT TAGAGCTTGG TTTTGCTTGG | 1500 |
| CTTGATTCAC ATACCCATTC TAGACTTTGG CATAAATTTG ATACGATAGA GAGTATCTAA | 1560 |
| TGGTAATGCA GAAGGGTAAA AAAAGGAAGA GAGAAAAGGT GAGAAAGATT ACCAAAAATA | 1620 |
| AGGAGTTTCA AAAGATGGTT CTGATGAGAA ACAGAGCCCA TCCCTCTCCT TTTCCCCTTC | 1680 |
| CCATGAAAGA AATCGGATGG TCCTCCTTCA ATGTCCTCCA CCTACTCTTC TCTTCTTTCT | 1740 |
| TTTTTTCTTT CTTATTATTA ACCATTTAAT TAATTTCCCC TTCAATTTCA GTTTCTAGTT | 1800 |
| CTGTAAAAAG AAAATACACA TCTCACTTAT AGATATCCAT ATCTATTTAT ATGCATGTAT | 1860 |
| AGAGAATAAA AAAGTGTGAG TTTCTAGGTA TGTTGAGTAT GTGCTGTTTG GACAATTGTT | 1920 |
| AGATGATCTG TCCATTTTTT TCTTTTTTCT TCTGTGTATA AATATATTTG AGCACAAAGA | 1980 |
| AAAACTAATA ACCTTCTGTT TTCAGCAACT AGGGTCTTAT AACCTTCAAA GAAATATTCC | 2040 |
| TTCAATTGAA AACCCATAAA CAAAATAGA TATTACAAAA GGAAAGAGAG ATATTTTCAA  | 2100 |
| GAACAACATA ATTAGAAAAG CAGAAGCAGC AGTTAAGTGG TACTGAGATA AATGATATAG | 2160 |
| TTTCTCTTCA AGAACAGTTT CTCATTACCC ACCTTCTCCT TTTTGCTGAT CTATCGTAAT | 2220 |
| CTTGAGAACT CAGGTAAGGT TGTGAATATT ATGCACCATT CATTAACCCT AAAAATAAGA | 2280 |
| GATTTAAAAT AAATGTTTCT TCTTTCTCTG ATTCTTGTGT AACCAATTCA TGGGTTTGAT | 2340 |
| ATGTTTCTTG GTTATTGCTT ATCAACAAAG AGATTTGATC ATTATAAAGT AGATTAATAA | 2400 |
| CTCTTAAACA CACAAAGTTT CTTTATTTTT TAGTTACATC CCTAATTCTA GACCAGAACA | 2460 |
| TGGATTTGAT CTATTTCTTG GTTATGTATC TTGATCAGGA AAAGGGATTT GATCATCAAG | 2520 |
| ATTAGCCTTC TCTCTCTCTC TCTAGATATC TTTCTTGAAT TTAGAAATCT TTATTTAATT | 2580 |
| ATTTGGTGAT GTCATATATG GATCAATGGA GGAAGGTGGG AGTAGTCACG ACGCAGAGAG | 2640 |
| TAGCAAGAAA CTAGGGAGAG GGAAAATAGA GATAAAGAGG ATAGAGAACA CAACAAATCG | 2700 |

|                                                                    |      |
|--------------------------------------------------------------------|------|
| TCAAGTTACT TTCTGCAAAC GACGCAATGG TCTTCTCAAG AAAGCTTATG AACTCTCTGT  | 2760 |
| CTTGTGTGAT GCCGAAGTTG CCCTCGTCAT CTTCTCCACT CGTGGCCGTC TCTATGAGTA  | 2820 |
| CGCCAACAAC AGGTACGCTT CTCCTACTCT ATTTCTTGAT CTGTGTTTTCT TAATTTTAAC | 2880 |
| TAAACAAGAT CCTAGTTCAA ATGATAACAA AGTGGGGATT GAGAGCCAAG ATTAGGGTTT  | 2940 |
| GGTTAATTTA GAAAACCAGA TTTCACCTTG GTATACATTT AATATCTCTC TAGCTAGATT  | 3000 |
| TAGTACTCTC TCCTCTATAT ATGTGTGGGT GTGTGTGTAA GTGTGTATAT GTATGCAAAT  | 3060 |
| GCAAGAAGAA GAAGAAAAAG TTATCTTGTC TTCTCAAATT CTGATCAGCT TTGACCTTAG  | 3120 |
| TTTCACCTCT TTTTCTGCAA ATCATTTGAA CCTGATGCAT GTCAGTTTCT ACAATACACT  | 3180 |
| TTTAATTTTG ACGGCCCATC AAATTTCCCTA GGGTTTACTT CAGTGAACAA AATTGGGTTC | 3240 |
| TTGACACGAT TTAGCATGTA TATATAAAAA TAGGGGATGA TCAAGACTTA TGTAACCTCT  | 3300 |
| GTCTGGTGAA ACTAGGGACA AAGTCTACTG ATGAGTTGTC ACTAGGGATC CATTTGATCA  | 3360 |
| TTTAATCCCA AAAAAAATGA AACAAAATTT TGAGAATTTA TATGCTGAAG TTTTCAACC   | 3420 |
| CTCTTTTTTA AATAACTTTA TATTATGTAG ATTTGTATTT AGGGTAATTT GTCCAACCTAG | 3480 |
| AAGTCCTAAA AATCAATAAA CACACGGATG ACTTTGTCTA ACATTGTATC AGTCATCAA   | 3540 |
| TGTAAAATTG TACAAATAAT GAAATTAAAG ATTTAGTCTC TTTTATTTTT TTTGTTTAGG  | 3600 |
| GTGTATATAT ATATATATAT GTATATTTGT TGCATTGATA TATCAATGAG AGGGAGAGAA  | 3660 |
| CTCAGAGAAG TGTCGGAAAT TAAAATGGTA CGAGCCAATT GGAATCTCTG GCATTCTGAG  | 3720 |
| CTTCATTTGT TTGTTATTAG AAAAAAAAAA AAAAAATCCT TTAAAGATAC CTTCATGATG  | 3780 |
| ACATTGAATC ATGTAATATA CACGATACAT GGTCTAATTC CTCCTCAAAC CCTAATTACC  | 3840 |
| AATTTGAAA CCATAATATT TACTAGTATG TTTATATATC CTTACTTTAA GACATTGTTT   | 3900 |
| GTTTATAATA CCTTGTGAAT TAAGAAAAAA AAAAAAAAC TTGTGGATCT ATTCAAGCCA   | 3960 |
| TGTGTTAGAA TAAATTTATA AATTTTCTCC TCGTACTGGT CAGATATTGG TCCAAACTCC  | 4020 |
| AAAGCCTTCC CTTTTCAGGA AAAAAACAT TTCGAAATTA ACTCTAATTA ATCAAGAATT   | 4080 |
| TCCTACAATG TATACATCTA ATGTTTTTTC CGCGATCTTA CTTATTAGTG TGAGGGGTAC  | 4140 |
| AATTGAAAGG TACAAGAAAG CTTGTTCCGA TGCCGTCAAC CCTCCTTCCG TCACCGAAGC  | 4200 |
| TAATACTCAG GTACCAATTT ATATTGTTTG ATTCTCTTTG TTTTATCTTC TTCTTTTCAT  | 4260 |
| TATATATATG ATCAACAAAA AATATAACCT AAAAAAGAG AGAGTTCAAG GAAATGCATT   | 4320 |
| GAAACGGTTT CGTTATGGTG TTTGAATACA TGGATTTTTG AAGTACTATC AGCAAGAAGC  | 4380 |
| CTCTAAGCTT CGGAGGCAGA TTCGAGATAT TCAGAATTCA AATAGGTAAT TCATTAAGTT  | 4440 |
| TTCATGAACT CTTGATTTG GTATTAGGTC ACTTAATTTG GTGTCGGTCC AAAAGTCCGC   | 4500 |
| TTGTAGTTTT CTTTAGAAGT TGTTTTGTTT AATGTTTATG TTTACAAATT GAAGGCATAT  | 4560 |
| TGTTGGGGAA TCACTTGGTT CCTTGAAGTT CAAGGAACTC AAAAACCTAG AAGGACGTCT  | 4620 |

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|                                                                   |      |
|-------------------------------------------------------------------|------|
| TGAAAAAGGA ATCAGCCGTG TCCGCTCCAA AAAGGTAAAA TCTACGTTGC TCTCTCTCTG | 4680 |
| TGTCTCTGTC TCTCTCTCTA TATATAGTCC CTTAGTTTAT ATAGTTCATC ACCCTTTTGT | 4740 |
| GAGAATTTTG CAGAATGAGC TGTTAGTGGC AGAGATAGAG TATATGCAGA AGAGGGTAAG | 4800 |
| AACGTTTCTC CCATTCCAAG TAATTAGATC TTTCTTCGTC TTTGTGAGGG TTTGAGTTTT | 4860 |
| CCCATAAATC ATGTGTAGGA AATGGAGTTG CAACACAATA ACATGTACCT GCGAGCAAAG | 4920 |
| GTTAGCCACG TTCTGTTCCA AATCTTAATC TCAATATCTA CTCTTTTCTT CATTGTATAA | 4980 |
| CTAAGATAAC GTGAATAACA AGAAACTTT TGTTTTTGGG TTTAATAGAT AGCCGAAGGC  | 5040 |
| GCCAGATTGA ATCCGGACCA GCAGGAATCG AGTGTGATAC AAGGGACGAC AGTTTACGAA | 5100 |
| TCCGGTGTAT CTTCTCATGA CCAGTCGCAG CATTATAATC GGAACATAT TCCGGTGAAC  | 5160 |
| CTTCTTGAAC CGAATCAGCA ATTCTCCGGC CAAGACCAAC CTCCTCTTCA ACTTGTGTAA | 5220 |
| CTCAAAACAT GATAACTTGT TTCTTCCCCT CATAACGATT AAGAGAGAGA CGAGAGAGTT | 5280 |
| CATTTTATAT TTATAACGCG ACTGTGTATT CATAGTTTAG GTTCTAATAA TGATAATAAC | 5340 |
| AAAACTGTTG TTTCTTTGCT TAATTACATC AACATTTAAA TCCAAAGTTC TAAAACACGT | 5400 |
| CGAGATCCAA AGTTTGTCAT ACAAGATTAG ACGCATAAC GATCAGTTAA TAGATTTTAA  | 5460 |
| GTGCCTTTTA ATATTTACAT ATAGTTGCAG CTTGATTAG ATCATGTCCA CCAAACACTC  | 5520 |
| ACAATTAGAG ACAAGCAAAA CTATAACAT TGATCATAAA ATGATTACAA CATGTCCATA  | 5580 |
| AATTAATTAT GGATTACAAA AATAAAACT TACAAAAGAT CT                     | 5622 |

(2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6138 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 1..6138
- (D) OTHER INFORMATION: /label= AGL5\_promoter  
/note= "Nucleotide sequence of the AGL5 promoter."

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| GAATTCGTAA CAGAATTTAG TGAATAATAT TGTAATTACC AGGCAAGGAC TCTCCAAACG | 60  |
| GATAGCTCGA ATATCGTTAT TAAAGAGTAA ATGATCCAAT ATGTAAGCCA TTGTTGATCA | 120 |
| TCTAACATTG TTGGACTCTC TATTGCTCGA AATGATGCAT ACCTAATCAT TTATTCAGTT | 180 |
| AACTATCAAG TTGCATTTGT AAAAACCAAA CATTTAAATT CAGATTTGAT ATCACTTACA | 240 |
| GAGGATAGAG AAGCATGACT CCAGGCCTGC ATGCAACAAG AAAAAGGAAG AAAATAATGT | 300 |
| TAAAAATTTG ACAAATATAG TGTTTATTTT TATTATATGA GACAGAATTT GAATAAAATC | 360 |

|                                                                   |      |
|-------------------------------------------------------------------|------|
| CTACCCAACT AGAGCATCAA AACGTTTTGC AATCGCAATA ATGAAACCCA TTTTCTTTTT | 420  |
| GAGTTTTTAC TCTTCTTTCA ACAGAACTT TCTCAAACGT CTTTAGCACT GTGACGTTAG  | 480  |
| ATATATACAC AAAAGCTTGA AATTTCTTCA AGCAAAAGAA TCTTTGTGGG AGTTAAGGCA | 540  |
| ACAAGCCAGG TAAAGAATCT CCAACGCATT GTTACGTTTT CATGAACCTA TTTATTATAT | 600  |
| GTTCTAAGAA AGAAAAAAT ATCTCAAAGT AAACGTTGGA AATTTTCTGA TGAAGGGAAA  | 660  |
| TCCAAAGTCT TGGGTTTAGT ATCCCTATGA ATGGTATTTG GAATATGTTT TCGTCAAAAC | 720  |
| AAAAGATTCT TTTCTTTTTT ACAAGAGTTA GTGATCAATA ACTTATGCAC TAATTAATGA | 780  |
| GATTGGACGT ATACACAATT TGATTATGAT ACTTGAGTAA AAATCACCTG TCCTTTAATT | 840  |
| TGGAAATCTC TCTTTCTTAC CCATTTATAT ACTACTTCTT TTCATTAAAA TTAAATTTCA | 900  |
| ATTATCAATC ATCGTTCAAT TTGATAAAGA TTTAACATTT TTTGTCACAG GGCTAGTAAA | 960  |
| AGCAATCTTT ACATAATTCA TCTTTCTTAC ATATATATAT TACCTTTTTT TCATTAGTA  | 1020 |
| TTCTATTTGA TTATGATTAT TTTGTCATAA AGCTAGTAAA TTAAACACTC GATATGAGAA | 1080 |
| TTATATTACT TCACGCTAAT TAACTCTTAA CACAACAAGA ACTAGTGCAT ATTCAACTTT | 1140 |
| CAAAGCATAT ACTATATATT GAGAATATAG ACCACGAAAG TCAATCAAAA GACCTACCAG | 1200 |
| CTCTCATCAA GTTCTTTCTT GAAATGATTT TGCAGAATTT CCAACTTAAT TAATTGACA  | 1260 |
| TGAATGTGAA AATGTGTGTT GCTCGTTAAG AAAATTGAAT AGAAGTACAA TGAAAATGAT | 1320 |
| GAGGAATGGG CAAAACACAA AAGAGTTTCC TTTCGTAAC ACATTAATT AATGCAAATC   | 1380 |
| TGAGAAAGGG TTCATGGATA ATGACTACAC ACATGATTAG TCATTCCCCG TGGGCTCTCT | 1440 |
| GCTTTCATTT ACTTTATTAG TTTCATCTTC TCTAATTATA TTGTCGCATA TATGATGCAG | 1500 |
| TTCTTTTGTC TAAATTACGT AATATGATGT AATTAATTAT CAAAATAAAT ATTCAAATTG | 1560 |
| CCGTTGGACT AACCTAATGT CCAAGATTAA GACTTGAACA TAAGAATTTT GGAAAACTA  | 1620 |
| AACCAGTTAT AATATATACT CTAAATTGC CATTTCTGAA CACAACCAA TAATAATATA   | 1680 |
| TACTATTTAC AGTTTTTTTT AATTGGCAAG AACACTGAAA TCTTATTCAT TGTCTCGCTT | 1740 |
| GGTAGTTGAC AAGTTATAAC ACTCATATTC ATATAACCCC ATTCTAACGT TGACGACGAA | 1800 |
| CACTCATATA AACCACCCAA ATTCTTAGCA TATTAGCTAA ATATTGGTTT AATTGGAAAT | 1860 |
| ATTTTTTTTA TATATAAAT GCCAGGTAAA TATTAACGAC ATGCAATGTA TATAGGAGTA  | 1920 |
| GGGCAATAAA AAGAAAAGGA GAATAAAAAG GGATTACCAA AAAAGGAAAG TTTCCAAAAG | 1980 |
| GTGATTCTGA TGAGAAACAG AGCCCATACC TCTCTTTTTT CCTCTAAACA TGAAAGAAAA | 2040 |
| ATTGGATGGT CCTCCTTCAA TGCTCTCTCC CCACCCAATC CAAACCCAAC TGTCTTCTTT | 2100 |
| CTTCTTTTTT TCTTCTTTCT AATTGATAT TTTCTACCAC TTAATTCCAA TCAATTTCAA  | 2160 |
| ATTTCAATCT AAATGTATGC ATATAGAATT TAATTAAAAG AATTAGGTGT GTGATATTTG | 2220 |
| AGAAAATGTT AGAAGTAATG GTCCATGTTC TTTCTTTCTT TTCCTTCTA TAACACTTCA  | 2280 |

|             |            |            |            |            |            |      |
|-------------|------------|------------|------------|------------|------------|------|
| GTTTGAAAAA  | AAACTACCAA | ACCTTCTGTT | TTCTGCAAAT | GGGTTTTTAA | ATACTTCCAA | 2340 |
| AGAAATATTC  | CTCTAAAAGA | AATTATAAAC | CAAAACAGAA | ACCAAAAACA | AAAAATAAAG | 2400 |
| TTGAAGCAGC  | AGTTAAGTGG | TACTGAGATA | ATAAGAATAG | TATCTTTAGG | CCAATGAACA | 2460 |
| AATTAACCTCT | CTCATAATTC | ATCTTCCCAT | CCTCACTTCT | CTTTCTTTCT | GATATAATTA | 2520 |
| ATCTTGCTAA  | GCCAGGTATG | GTTATTGATG | ATTTACACTT | TTTTTTAAAA | GTTTCTTCCT | 2580 |
| TTTCTCCAAT  | CAAATTCTTC | AGTTAATCCT | TATAAACCAT | TTCTTTAATC | CAAGGTGTTT | 2640 |
| GAGTGCAAAA  | GGATTTGATC | TATTTCTCTT | GTGTTTATAC | TTCAGCTAGG | GCTTATAGAA | 2700 |
| ATGGAGGCTG  | GTGCGAGTAA | TGAAGTAGCA | GAGAGCAGCA | AGAAGATAGG | GAGAGGGAAG | 2760 |
| ATAGAGATAA  | AGAGGATAGA | GAACACTACG | AATCGTCAAG | TCACTTTCTG | CAAACGACGC | 2820 |
| AATGGTTTAC  | TCAAGAAAGC | TTATGAGCTC | TCTGTCTTGT | GTGACGCTGA | GGTTGCTCTT | 2880 |
| GTCATCTTCT  | CCACTCGAGG | CCGTCTCTAC | GAGTACGCCA | ACAACAGGTA | CACATCTTTT | 2940 |
| AGCTAGATCT  | TGATTTTGTT | GAATTTTTTT | TCTAGAATAA | AGTTTCGACT | CTTCTGGTGG | 3000 |
| GTTTTTCAAT  | CTTTATGGTC | TCTTTATAGT | TTTTTTCCTT | ACTTTCTCTG | AAGCTCAAAT | 3060 |
| CTCTTTAAAA  | ATCCCCAAAA | TTAGGGTTTG | TTTAAACTA  | GGGAACCCTA | CTTTAACTTC | 3120 |
| TTTCTCTTAG  | TAAAAAAGCA | GTGAGGGTCT | TCTCTGATCA | TTAATTAGCA | TCCCCCATA  | 3180 |
| CTTGTTCCAG  | TCACTTTTTC | TCCACAAATC | CTTATAACAG | TATCTATATA | TGTATCTATT | 3240 |
| TATGTCAGTT  | TGTACAAGAC | ACTTCGATCA | ATTTGATGAC | CCATCAAGTT | TTATTTCTGC | 3300 |
| AGATTGATCA  | TTAGGTTTCC | ATCATAGTAA | TGAAAAAGTA | GGGTTCTTGA | TAAAATTATA | 3360 |
| ATAATATATA  | TTATTTGGCT | ATATAAAAAA | GCTATGTAGA | TTCCTTAAAA | ATTGATTCAC | 3420 |
| TAGGGAGAGA  | CTAGTAGGTG | TTTGTCTTCT | GACACTTCTC | TAATCTTTTG | GTGAATCCTT | 3480 |
| TTGTTAAATC  | AAGAAAATGA | ATCAGGGACA | AAGCTTATTG | TTGAGTCACT | TAATTAATCA | 3540 |
| TCCGATCCAT  | CAATCAAGAA | AAATAACGAA | ACAGAAAATT | TTGATTTTTG | ATTGTTATTT | 3600 |
| TCTCCACTTC  | AAGTTGGGGA | CTTGTCATTT | CCGTTTTTCT | ATACGTTTCC | AGCTATTAAC | 3660 |
| AGCTCATGTT  | CATTTACCA  | TTTTGATTAT | TTGTCTGCTT | TTTAAAGATA | AATGTTTTCA | 3720 |
| AAAATATTGT  | TTTTATTTGC | TTGGCTAGTT | AATACTATAA | TTGAGGTGTA | TGTATGACTA | 3780 |
| TAATCTATAA  | GTCAAGTCTC | ATATCATGGA | TCTAAGTTAA | AACTAGTAAA | TTTGTAGTTT | 3840 |
| CAATGTGAAC  | TTTACAACG  | ACTAAAGAAC | TGATCTGAAG | TTTATAATGG | ACATGACTAA | 3900 |
| TTTGATTAAC  | AAAAGAGGAA | TGCATTATGT | ATGTAGAAAC | ATGTGATATA | TATATGTTTC | 3960 |
| TATTATCAAA  | AGTGTAGTTA | ACTTTCTTAT | TTCAAACACC | CTCATGCTTT | AGTAGTATCT | 4020 |
| TACTTTTGAC  | ATTTCTCAAC | TTCAGCTTTC | CATTATACAA | CAGCACAATG | TAAATTACTT | 4080 |
| GATATGAAT   | ATGAAAGCAT | AACGTTATGC | AAAGATTTCT | AGCTTTTCTT | TTTCTGTTTT | 4140 |
| GCAAAAGATT  | TACAAATATC | ATGTTCTTGG | TAAAAACATA | CTTGCCTCAG | CCACATATGC | 4200 |



|                                                                   |      |
|-------------------------------------------------------------------|------|
| ATGTAAATGT AATGTTCAAA TATTAATTCA GGAAAAACAA AGAAGAAGCA AAATTAGCTT | 4260 |
| CTAGAGTAGG GAATCTATTG ACTTGACCTG AAAATCACTT CTTTTTCTTA AAGCCTAGTA | 4320 |
| GTGAATTTTT TAATCTAATT AGGCCAAAAT ATATACTAGC CTAAAATATA ATTTGGATTT | 4380 |
| TGTGTCGTAC ATAAATTGGG ACCAATTCCA ATTAATAAG AGCATATGCA ATTCAAATTC  | 4440 |
| TTTTTATTTT CTTCTCCGAT TTGCTACTTC TTTCTTTTGT ATGTTTTCAA ATTAGGATTA | 4500 |
| CACTTTTTTG GGAAGTACA CATTAGGGTC TTCTCGAACT TTGATTATAC ATATATATAT  | 4560 |
| ATATATATAT ATATAACTTT GTGAGATGTC ACTGTTAATA GATAATAGGC AATAACAATA | 4620 |
| ATATCCAAAA AAGAAGGCGC AAACAAATCA TATACTATAT GGTACTGGTC CATTCACTAT | 4680 |
| TTTGTCGGTT GAATTTAAGG TTTGGCGTAC AAACTTTGTT TCAAACCTTT ATTATTCCGT | 4740 |
| CTTTCTGTGT GTTTTGTATA TCCAGAAGAT AAAAATATCA ATTTCTTTAA CGACTTCATA | 4800 |
| TATATATATA TATATATATA TATATATATT TTTCTCTTCT GGTTTTAGTG TTTGAATCCA | 4860 |
| ACAGTTATAG TTTCGTGTGT CTTTGTTTTA CTTGTGGTGG TTTAAGTTTG AGATTTTCAC | 4920 |
| CGATTGCATC TATTTACATA TATAGCTACC AAAAAAAGA TTGCATTTTA AAATCTTTTC  | 4980 |
| CTTTGTGTGA ATGTTGATGA AGTGTGAGAG GAACAATAGA AAGGTACAAG AAAGCTTGCT | 5040 |
| CCGACGCCGT TAACCCTCCG ACCATCACCG AAGCTAATAC TCAGGTTAGC TTTTAATTAA | 5100 |
| TACACCTAGC TAGCTAGTTC GTTAATTACT TAATTTCTTC TTCTTTTAGT TATCTGACCT | 5160 |
| TTTTTTCACC TCTTGTAACA ATGATGGGAT CGAAATTGAT GAAGTACTAT CAGCAAGAGG | 5220 |
| CGTCTAAACT CCGGAGACAG ATTCGGGACA TTCAGAATTT GAACAGACAC ATTCTTGGTG | 5280 |
| AATCTCTTGG TTCCTTGAAC TTTAAGGAAC TCAAGAACCT TGAAAGTAGG CTTGAGAAAG | 5340 |
| GAATCAGTCG TGTCCGATCC AAGAAGGTAC ATCACTAACT CTCCATCAAT CTCCTTATCA | 5400 |
| TTGAATATAT ATCCATCTGA TTCTTGCCCG TTATATTTGG TTTTCTCTC CAGCACGAGA  | 5460 |
| TGTTAGTTGC AGAGATTGAA TACATGCAAA AAAGGGTAAA AGTAAAACCT ATCTTCCTTC | 5520 |
| ACAATGAACT ACCCCTACTT TATTAGCAAC TTCTCTTTCT GATGATCATC TTTTTTATTT | 5580 |
| TCTGTTGTCG CTTGCATTGT AGGAAATCGA GCTGCAAAAC GATAACATGT ATCTCCGCTC | 5640 |
| CAAGGTTTTA TACATAACTC TTTTGGCAT TTTTGATCAT CATTTTTTTC CGGTAGACAA  | 5700 |
| TCTCTTGATG TGCAAATTCT AAATATCTCT GCAGATTACT GAAAGAACAG GTCTACAGCA | 5760 |
| ACAAGAATCG AGTGTGATAC ATCAAGGGAC AGTTTACGAG TCGGGTGTTA CTTCTTCTCA | 5820 |
| CCAGTCGGGG CAGTATAACC GGAATTATAT TGCGGTTAAC CTTCTTGAAC CGAATCAGAA | 5880 |
| TTCCTCCAAC CAAGACCAAC CACCTCTGCA ACTTGTTTGA TTCAGTCTAA CATAAGCTTC | 5940 |
| TTTCCTCAGC CTGAGATCGA TCTATAGTGT CACCTAAATG CGGCCGCGTC CCTCAACATC | 6000 |
| TAGTCGCAAG CTGAGGGGAA CCACTAGTGT CATACGAACC TCCAAGAGAC GGTTACACAA | 6060 |
| ACGGGTACAT TGTTGATGTC ATGTATGACA ATCGCCCAAG TAAGTATCCA GCTGTGTTCA | 6120 |



GAACGTACGT CCGAATTC

6138

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 896 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 7..753

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 896
- (D) OTHER INFORMATION: /note= "There is a poly(A) tail at the end of the cDNA sequence."

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 1..896
- (D) OTHER INFORMATION: /note= "AGL1 cDNA and deduced protein sequences."

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

|                                                                 |     |
|-----------------------------------------------------------------|-----|
| GGATCA ATG GAG GAA GGT GGG AGT AGT CAC GAC GCA GAG AGT AGC AAG  | 48  |
| Met Glu Glu Gly Gly Ser Ser His Asp Ala Glu Ser Ser Lys         |     |
| 1 5 10                                                          |     |
| AAA CTA GGG AGA GGG AAA ATA GAG ATA AAG AGG ATA GAG AAC ACA ACA | 96  |
| Lys Leu Gly Arg Gly Lys Ile Glu Ile Lys Arg Ile Glu Asn Thr Thr |     |
| 15 20 25 30                                                     |     |
| AAT CGT CAA GTT ACT TTC TGC AAA CGA CGC AAT GGT CTT CTC AAG AAA | 144 |
| Asn Arg Gln Val Thr Phe Cys Lys Arg Arg Asn Gly Leu Leu Lys Lys |     |
| 35 40 45                                                        |     |
| GCT TAT GAA CTC TCT GTC TTG TGT GAT GCC GAA GTT GCC CTC GTC ATC | 192 |
| Ala Tyr Glu Leu Ser Val Leu Cys Asp Ala Glu Val Ala Leu Val Ile |     |
| 50 55 60                                                        |     |
| TTC TCC ACT CGT GGC CGT CTC TAT GAG TAC GCC AAC AAC AGT GTG AGG | 240 |
| Phe Ser Thr Arg Gly Arg Leu Tyr Glu Tyr Ala Asn Asn Ser Val Arg |     |
| 65 70 75                                                        |     |
| GGT ACA ATT GAA AGG TAC AAG AAA GCT TGT TCC GAT GCC GTC AAC CCT | 288 |
| Gly Thr Ile Glu Arg Tyr Lys Lys Ala Cys Ser Asp Ala Val Asn Pro |     |
| 80 85 90                                                        |     |
| CCT TCC GTC ACC GAA GCT AAT ACT CAG TAC TAT CAG CAA GAA GCC TCT | 336 |
| Pro Ser Val Thr Glu Ala Asn Thr Gln Tyr Tyr Gln Gln Glu Ala Ser |     |
| 95 100 105 110                                                  |     |

|                                                                                                                                                       |     |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| AAG CTT CGG AGG CAG ATT CGA GAT ATT CAG AAT TCA AAT AGG CAT ATT<br>Lys Leu Arg Arg Gln Ile Arg Asp Ile Gln Asn Ser Asn Arg His Ile<br>115 120 125     | 384 |
| GTT GGG GAA TCA CTT GGT TCC TTG AAC TTC AAG GAA CTC AAA AAC CTA<br>Val Gly Glu Ser Leu Gly Ser Leu Asn Phe Lys Glu Leu Lys Asn Leu<br>130 135 140     | 432 |
| GAA GGA CGT CTT GAA AAA GGA ATC AGC CGT GTC CGC TCC AAA AAG AAT<br>Glu Gly Arg Leu Glu Lys Gly Ile Ser Arg Val Arg Ser Lys Lys Asn<br>145 150 155     | 480 |
| GAG CTG TTA GTG GCA GAG ATA GAG TAT ATG CAG AAG AGG GAA ATG GAG<br>Glu Leu Leu Val Ala Glu Ile Glu Tyr Met Gln Lys Arg Glu Met Glu<br>160 165 170     | 528 |
| TTG CAA CAC AAT AAC ATG TAC CTG CGA GCA AAG ATA GCC GAA GGC GCC<br>Leu Gln His Asn Asn Met Tyr Leu Arg Ala Lys Ile Ala Glu Gly Ala<br>175 180 185 190 | 576 |
| AGA TTG AAT CCG GAC CAG CAG GAA TCG AGT GTG ATA CAA GGG ACG ACA<br>Arg Leu Asn Pro Asp Gln Gln Glu Ser Ser Val Ile Gln Gly Thr Thr<br>195 200 205     | 624 |
| GTT TAC GAA TCC GGT GTA TCT TCT CAT GAC CAG TCG CAG CAT TAT AAT<br>Val Tyr Glu Ser Gly Val Ser Ser His Asp Gln Ser Gln His Tyr Asn<br>210 215 220     | 672 |
| CGG AAC TAT ATT CCG GTG AAC CTT CTT GAA CCG AAT CAG CAA TTC TCC<br>Arg Asn Tyr Ile Pro Val Asn Leu Leu Glu Pro Asn Gln Gln Phe Ser<br>225 230 235     | 720 |
| GGC CAA GAC CAA CCT CCT CTT CAA CTT GTG TAACTCAAAA CATGATAACT<br>Gly Gln Asp Gln Pro Pro Leu Gln Leu Val<br>240 245                                   | 770 |
| TGTTTCTTCC CCTCATAACG ATTAAGAGAG AGACGAGAGA GTTCATTTTA TATTTATAAC                                                                                     | 830 |
| GCGACTGTGT ATTCATAGTT TAGGTTCTAA TAATGATAAT AACAAAAC TG TTGTTTCTTT                                                                                    | 890 |
| GCTTCA                                                                                                                                                | 896 |

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 248 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

|                                                                              |
|------------------------------------------------------------------------------|
| Met Glu Glu Gly Gly Ser Ser His Asp Ala Glu Ser Ser Lys Lys Leu<br>1 5 10 15 |
| Gly Arg Gly Lys Ile Glu Ile Lys Arg Ile Glu Asn Thr Thr Asn Arg<br>20 25 30  |
| Gln Val Thr Phe Cys Lys Arg Arg Asn Gly Leu Leu Lys Lys Ala Tyr<br>35 40 45  |

89

Glu Leu Ser Val Leu Cys Asp Ala Glu Val Ala Leu Val Ile Phe Ser  
 50 55 60  
 Thr Arg Gly Arg Leu Tyr Glu Tyr Ala Asn Asn Ser Val Arg Gly Thr  
 65 70 75 80  
 Ile Glu Arg Tyr Lys Lys Ala Cys Ser Asp Ala Val Asn Pro Pro Ser  
 85 90 95  
 Val Thr Glu Ala Asn Thr Gln Tyr Tyr Gln Gln Glu Ala Ser Lys Leu  
 100 105 110  
 Arg Arg Gln Ile Arg Asp Ile Gln Asn Ser Asn Arg His Ile Val Gly  
 115 120 125  
 Glu Ser Leu Gly Ser Leu Asn Phe Lys Glu Leu Lys Asn Leu Glu Gly  
 130 135 140  
 Arg Leu Glu Lys Gly Ile Ser Arg Val Arg Ser Lys Lys Asn Glu Leu  
 145 150 155 160  
 Leu Val Ala Glu Ile Glu Tyr Met Gln Lys Arg Glu Met Glu Leu Gln  
 165 170 175  
 His Asn Asn Met Tyr Leu Arg Ala Lys Ile Ala Glu Gly Ala Arg Leu  
 180 185 190  
 Asn Pro Asp Gln Gln Glu Ser Ser Val Ile Gln Gly Thr Thr Val Tyr  
 195 200 205  
 Glu Ser Gly Val Ser Ser His Asp Gln Ser Gln His Tyr Asn Arg Asn  
 210 215 220  
 Tyr Ile Pro Val Asn Leu Leu Glu Pro Asn Gln Gln Phe Ser Gly Gln  
 225 230 235 240  
 Asp Gln Pro Pro Leu Gln Leu Val  
 245

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 959 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 78..818

## (ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 1..959
- (D) OTHER INFORMATION: /note= "AGL5 cDNA and deduced protein sequences."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| GAATTCATCT TCCCATCCTC ACTTCTCTTT CTTTCTGATC ATAATTAATC TTGCTAAGCC | 60  |
| AGCTAGGGCT TATAGAA ATG GAG GGT GGT GCG AGT AAT GAA GTA GCA GAG    | 110 |
| Met Glu Gly Gly Ala Ser Asn Glu Val Ala Glu                       |     |
| 1 5 10                                                            |     |
| AGC AGC AAG AAG ATA GGG AGA GGG AAG ATA GAG ATA AAG AGG ATA GAG   | 158 |
| Ser Ser Lys Lys Ile Gly Arg Gly Lys Ile Glu Ile Lys Arg Ile Glu   |     |
| 15 20 25                                                          |     |
| AAC ACT ACG AAT CGT CAA GTC ACT TTC TGC AAA CGA CGC AAT GGT TTA   | 206 |
| Asn Thr Thr Asn Arg Gln Val Thr Phe Cys Lys Arg Arg Asn Gly Leu   |     |
| 30 35 40                                                          |     |
| CTC AAG AAA GCT TAT GAG CTC TCT GTC TTG TGT GAC GCT GAG GTT GCT   | 254 |
| Leu Lys Lys Ala Tyr Glu Leu Ser Val Leu Cys Asp Ala Glu Val Ala   |     |
| 45 50 55                                                          |     |
| CTT GTC ATC TTC TCC ACT CGA GGC CGT CTC TAC GAG TAC GCC AAC AAC   | 302 |
| Leu Val Ile Phe Ser Thr Arg Gly Arg Leu Tyr Glu Tyr Ala Asn Asn   |     |
| 60 65 70 75                                                       |     |
| AGT GTG AGA GGA ACA ATA GAA AGG TAC AAG AAA GCT TGC TCC GAC GCC   | 350 |
| Ser Val Arg Gly Thr Ile Glu Arg Tyr Lys Lys Ala Cys Ser Asp Ala   |     |
| 80 85 90                                                          |     |
| GTT AAC CCT CCG ACC ATC ACC GAA GCT AAT ACT CAG TAC TAT CAG CAA   | 398 |
| Val Asn Pro Pro Thr Ile Thr Glu Ala Asn Thr Gln Tyr Tyr Gln Gln   |     |
| 95 100 105                                                        |     |
| GAG GCG TCT AAA CTC CGG AGA CAG ATT CGG GAC ATT CAG AAT TTG AAC   | 446 |
| Glu Ala Ser Lys Leu Arg Arg Gln Ile Arg Asp Ile Gln Asn Leu Asn   |     |
| 110 115 120                                                       |     |
| AGA CAC ATT CTT GGT GAA TCT CTT GGT TCC TTG AAC TTT AAG GAA CTC   | 494 |
| Arg His Ile Leu Gly Glu Ser Leu Gly Ser Leu Asn Phe Lys Glu Leu   |     |
| 125 130 135                                                       |     |
| AAG AAC CTT GAA AGT AGG CTT GAG AAA GGA ATC AGT CGT GTC CGA TCC   | 542 |
| Lys Asn Leu Glu Ser Arg Leu Glu Lys Gly Ile Ser Arg Val Arg Ser   |     |
| 140 145 150 155                                                   |     |
| AAG AAG CAC GAG ATG TTA GTT GCA GAG ATT GAA TAC ATG CAA AAA AGG   | 590 |
| Lys Lys His Glu Met Leu Val Ala Glu Ile Glu Tyr Met Gln Lys Arg   |     |
| 160 165 170                                                       |     |
| GAA ATC GAG CTG CAA AAC GAT AAC ATG TAT CTC CGC TCC AAG ATT ACT   | 638 |
| Glu Ile Glu Leu Gln Asn Asp Asn Met Tyr Leu Arg Ser Lys Ile Thr   |     |
| 175 180 185                                                       |     |
| GAA AGA ACA GGT CTA CAG CAA CAA GAA TCG AGT GTG ATA CAT CAA GGG   | 686 |
| Glu Arg Thr Gly Leu Gln Gln Gln Glu Ser Ser Val Ile His Gln Gly   |     |
| 190 195 200                                                       |     |
| ACA GTT TAC GAG TCG GGT GTT ACT TCT TCT CAC CAG TCG GGG CAG TAT   | 734 |
| Thr Val Tyr Glu Ser Gly Val Thr Ser Ser His Gln Ser Gly Gln Tyr   |     |
| 205 210 215                                                       |     |

91

AAC CGG AAT TAT ATT GCG GTT AAC CTT CTT GAA CCG AAT CAG AAT TCC 782  
 Asn Arg Asn Tyr Ile Ala Val Asn Leu Leu Glu Pro Asn Gln Asn Ser  
 220 225 230 235  
 TCC AAC CAA GAC CAA CCA CCT CTG CAA CTT GTT TGATTCAGTC TAACATAAGC 835  
 Ser Asn Gln Asp Gln Pro Pro Leu Gln Leu Val  
 240 245  
 TTCTTTCCTC AGCCTGAGAT CGATCTATAG TGTCACCTAA ATGCGGCCGC GTCCCTCAAC 895  
 ATCTAGTCGC AAGCTGAGGG GAACCACTAG TGTCATACGA ACCTCCAAGA GACGGTTACA 955  
 CAAA 959

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 246 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Glu Gly Gly Ala Ser Asn Glu Val Ala Glu Ser Ser Lys Lys Ile  
 1 5 10 15  
 Gly Arg Gly Lys Ile Glu Ile Lys Arg Ile Glu Asn Thr Thr Asn Arg  
 20 25 30  
 Gln Val Thr Phe Cys Lys Arg Arg Asn Gly Leu Leu Lys Lys Ala Tyr  
 35 40 45  
 Glu Leu Ser Val Leu Cys Asp Ala Glu Val Ala Leu Val Ile Phe Ser  
 50 55 60  
 Thr Arg Gly Arg Leu Tyr Glu Tyr Ala Asn Asn Ser Val Arg Gly Thr  
 65 70 75 80  
 Ile Glu Arg Tyr Lys Lys Ala Cys Ser Asp Ala Val Asn Pro Pro Thr  
 85 90 95  
 Ile Thr Glu Ala Asn Thr Gln Tyr Tyr Gln Gln Glu Ala Ser Lys Leu  
 100 105 110  
 Arg Arg Gln Ile Arg Asp Ile Gln Asn Leu Asn Arg His Ile Leu Gly  
 115 120 125  
 Glu Ser Leu Gly Ser Leu Asn Phe Lys Glu Leu Lys Asn Leu Glu Ser  
 130 135 140  
 Arg Leu Glu Lys Gly Ile Ser Arg Val Arg Ser Lys Lys His Glu Met  
 145 150 155 160  
 Leu Val Ala Glu Ile Glu Tyr Met Gln Lys Arg Glu Ile Glu Leu Gln  
 165 170 175  
 Asn Asp Asn Met Tyr Leu Arg Ser Lys Ile Thr Glu Arg Thr Gly Leu  
 180 185 190

92

Gln Gln Gln Glu Ser Ser Val Ile His Gln Gly Thr Val Tyr Glu Ser  
 195 200 205

Gly Val Thr Ser Ser His Gln Ser Gly Gln Tyr Asn Arg Asn Tyr Ile  
 210 215 220

Ala Val Asn Leu Leu Glu Pro Asn Gln Asn Ser Ser Asn Gln Asp Gln  
 225 230 235 240

Pro Pro Leu Gln Leu Val  
 245

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 1..27
- (D) OTHER INFORMATION: /note= "Primer AGL8 5-4"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CCGTCGACGA TGGGAAGAGG TAGGGTT

27

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 1..20
- (D) OTHER INFORMATION: /note= "Primer OAM14."

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AATCATTACC AAGATATGAA

20

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear



(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CGGATAGCTC GAATATCG

18

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AACATTGCGT CGTTTGC

17

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTAATTACCA GGCAAGGACT CTCC

24

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GTCATCGGCG GGGGTCATAA CGTG

24

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GAGGATAGAG AACACTACGA ATCG

24

## (2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CAGGTCAAGT CAATAGATTC

20

## (2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 22 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CAGAATTTAG TGAATAATAT TG

22

## (2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GCCAGAGATA ATGCTATTCC

20

## (2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 23 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CATTGATCCA TATATGACAT CAC

23

## (2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 41 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

95

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GTGATGTCAT ATATGGATCA ATGGGAAGAG GTAGGGTTCA G

41

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CAAGAGTCGG TGGAATATTC G

21

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 41 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGAATATTCC ACCGACTCTT GGTACGCTTC TCCTACTCTA T

41

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CTAATAAGTA AGATCGCGGA A

21

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 41 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TTCCGCGATC TTAATTATTA GCATGGAGAG GATACTTGAA C

41

We claim:

1. A non-naturally occurring seed plant, comprising an ectopically expressed nucleic acid molecule encoding an AGL8-like gene product, said seed plant  
5 characterized by delayed seed dispersal.
2. The non-naturally occurring seed plant of claim 1, wherein said AGL8-like gene product has substantially the amino acid sequence of an AGL8 ortholog.
- 10 3. The non-naturally occurring seed plant of claim 2, wherein said AGL8-like gene product has the amino acid sequence of *Arabidopsis* AGL8 (SEQ ID NO:2).
4. The non-naturally occurring seed plant of claim 3, which is a transgenic seed plant.
- 15 5. The transgenic seed plant of claim 4, wherein said ectopically expressed nucleic acid molecule encoding an AGL8-like gene product is operatively linked to an exogenous regulatory element.
6. The transgenic seed plant of claim 5,  
20 wherein said exogenous regulatory element is a constitutive regulatory element.
7. The transgenic seed plant of claim 6, said nucleic acid molecule comprising an exogenous nucleic acid molecule encoding substantially the amino acid  
25 sequence of an AGL8 ortholog operatively linked to a cauliflower mosaic virus 35S promoter.

8. The transgenic seed plant of claim 5, wherein said exogenous regulatory element is a dehiscence zone-selective regulatory element.

9. The transgenic seed plant of claim 8,  
5 wherein said dehiscence zone-selective regulatory element is selected from the group consisting of an *AGL1* regulatory element and an *AGL5* regulatory element.

10. The transgenic seed plant of claim 9,  
10 wherein said nucleic acid molecule encoding an *AGL8*-like gene product is an exogenous nucleic acid molecule encoding substantially the amino acid sequence of an *AGL8* ortholog.

11. The transgenic seed plant of claim 10,  
15 wherein said *AGL8*-like gene product has the amino acid sequence of *Arabidopsis AGL8* (SEQ ID NO:2).

12. The transgenic seed plant of claim 9,  
wherein said dehiscence-zone selective regulatory element is an *AGL1* regulatory element comprising at least fifteen contiguous nucleotides of a nucleotide sequence selected  
20 from the group consisting of:

nucleotides 1 to 2599 of SEQ ID NO:3;

nucleotides 2833 to 4128 of SEQ ID NO:3;

nucleotides 4211 to 4363 of SEQ ID NO:3;

nucleotides 4426 to 4554 of SEQ ID NO:3;

25 nucleotides 4655 to 4753 of SEQ ID NO:3;

nucleotides 4796 to 4878 of SEQ ID NO:3;

nucleotides 4921 to 5028 of SEQ ID NO:3; and

nucleotides 5421 to 5682 of SEQ ID NO:3.

13. The transgenic seed plant of claim 9,  
wherein said dehiscence-zone selective regulatory element  
is an AGL5 regulatory element comprising at least fifteen  
contiguous nucleotides of a nucleotide sequence selected  
5 from the group consisting of:

nucleotides 1 to 1888 of SEQ ID NO:4;  
nucleotides 2928 to 5002 of SEQ ID NO:4;  
nucleotides 5085 to 5204 of SEQ ID NO:4;  
nucleotides 5367 to 5453 of SEQ ID NO:4;  
10 nucleotides 5496 to 5602 of SEQ ID NO:4;  
nucleotides 5645 to 5734 of SEQ ID NO:4; and  
nucleotides 6062 to 6138 of SEQ ID NO:4.

14. The non-naturally occurring seed plant of  
claim 1, which is a dehiscent seed plant.

15 15. The non-naturally occurring seed plant of  
claim 14, which is a member of the *Brassicaceae*.

16. The non-naturally occurring seed plant of  
claim 14, which is a member of the *Fabaceae*.

17. A non-naturally occurring seed plant, in  
20 which AGL1 expression and AGL5 expression each are  
suppressed, said seed plant characterized by delayed seed  
dispersal.

18. The non-naturally occurring seed plant of  
claim 17, which is an *agl1 agl5* double mutant.

19. A tissue derived from a non-naturally occurring seed plant, said seed plant comprising an ectopically expressible nucleic acid molecule encoding an AGL8-like gene product and characterized by delayed seed  
5 dispersal.

20. The tissue of claim 19, which is a seed.

21. A tissue derived from a non-naturally occurring seed plant, in which AGL1 expression and AGL5 expression each are suppressed, said seed plant  
10 characterized by delayed seed dispersal.

22. The tissue of claim 21, which is a seed.

23. A method of producing a non-naturally occurring seed plant characterized by delayed seed dispersal, comprising ectopically expressing a nucleic acid molecule encoding an AGL8-like gene product in said  
15 seed plant, whereby seed dispersal is delayed due to ectopic expression of said nucleic acid molecule.

24. A substantially purified dehiscence zone-selective regulatory element, comprising a  
20 nucleotide sequence that confers selective expression upon an operatively linked nucleic acid molecule in the valve margin or dehiscence zone of a seed plant,  
provided that said dehiscence zone-selective regulatory element does not have a nucleotide sequence  
25 consisting of nucleotides 1889 to 2703 of SEQ ID NO:4.



25. The substantially purified dehiscence zone-selective regulatory element of claim 24, which is selected from the group consisting of an *AGL1* regulatory element and an *AGL5* regulatory element.

5           26. The substantially purified dehiscence zone-selective regulatory element of claim 25, which is an *AGL1* regulatory element comprising at least fifteen contiguous nucleotides of a nucleotide sequence selected from the group consisting of:

10           nucleotides 1 to 2599 of SEQ ID NO:3;  
              nucleotides 2833 to 4128 of SEQ ID NO:3;  
              nucleotides 4211 to 4363 of SEQ ID NO:3;  
              nucleotides 4426 to 4554 of SEQ ID NO:3;  
              nucleotides 4655 to 4753 of SEQ ID NO:3;  
15           nucleotides 4796 to 4878 of SEQ ID NO:3;  
              nucleotides 4921 to 5028 of SEQ ID NO:3; and  
              nucleotides 5361 to 5622 of SEQ ID NO:3.

            27. The substantially purified dehiscence zone-selective regulatory element of claim 25, which is  
20           an *AGL5* regulatory element comprising at least fifteen contiguous nucleotides of a nucleotide sequence selected from the group consisting of:

              nucleotides 1 to 1888 of SEQ ID NO:4;  
              nucleotides 2928 to 5002 of SEQ ID NO:4;  
25           nucleotides 5085 to 5204 of SEQ ID NO:4;  
              nucleotides 5367 to 5453 of SEQ ID NO:4;  
              nucleotides 5496 to 5602 of SEQ ID NO:4;  
              nucleotides 5645 to 5734 of SEQ ID NO:4; and  
              nucleotides 6062 to 6138 of SEQ ID NO:4.

30           28. A plant expression vector, comprising a dehiscence zone-selective regulatory element.

29. A kit for producing a transgenic seed plant characterized by delayed seed dispersal, comprising a dehiscence zone-selective regulatory element having a nucleotide sequence that confers selective expression  
5 upon an operatively linked nucleic acid molecule in the valve margin or dehiscence zone of a seed plant,

provided that said dehiscence zone-selective regulatory element does not have a nucleotide sequence consisting of nucleotides 1889 to 2703 of SEQ ID NO:4.

10 30. The kit of claim 29, said dehiscence zone-selective regulatory element is operatively linked to a nucleic acid molecule encoding an AGL8-like gene product.

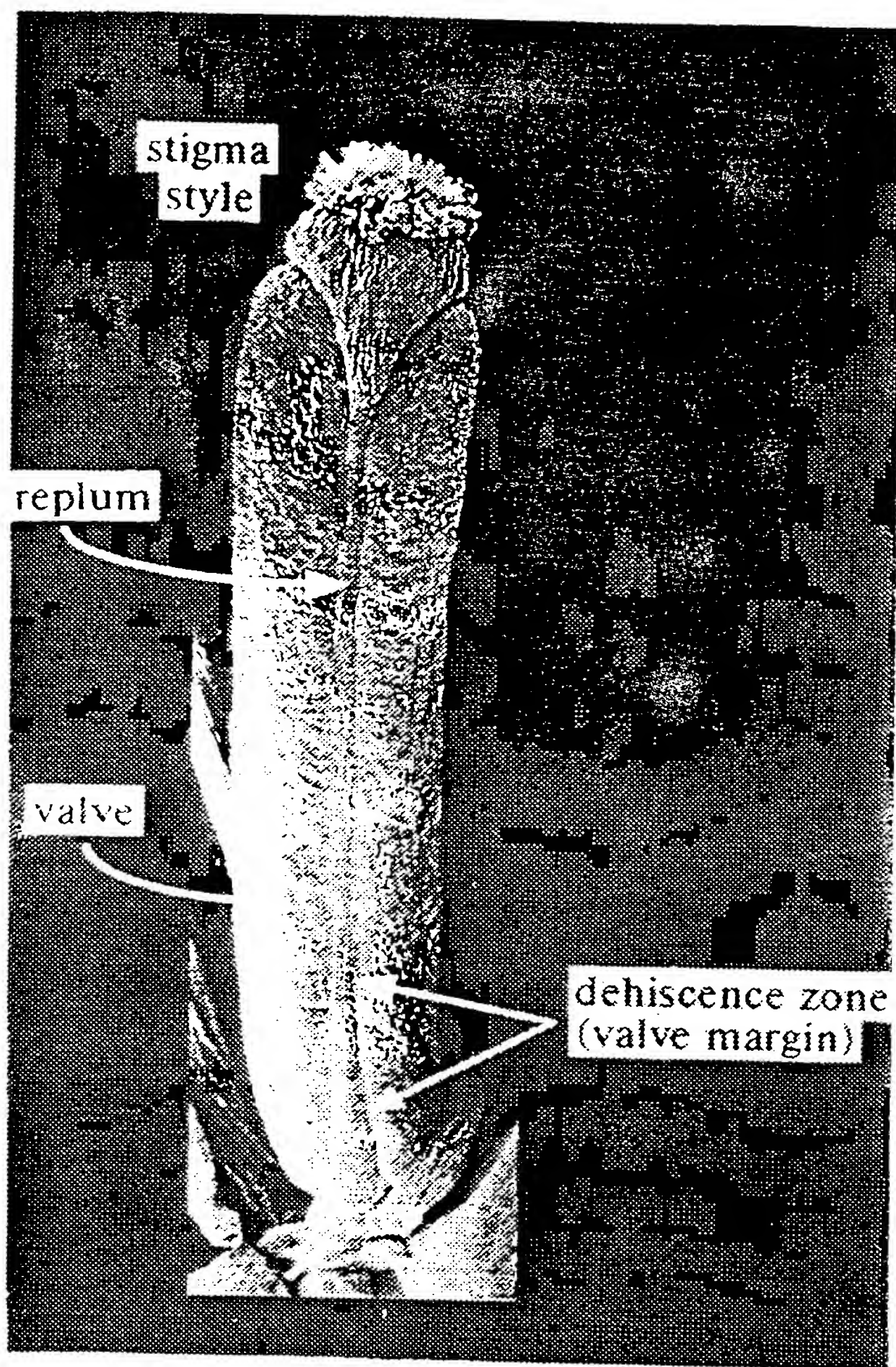


FIG. 1

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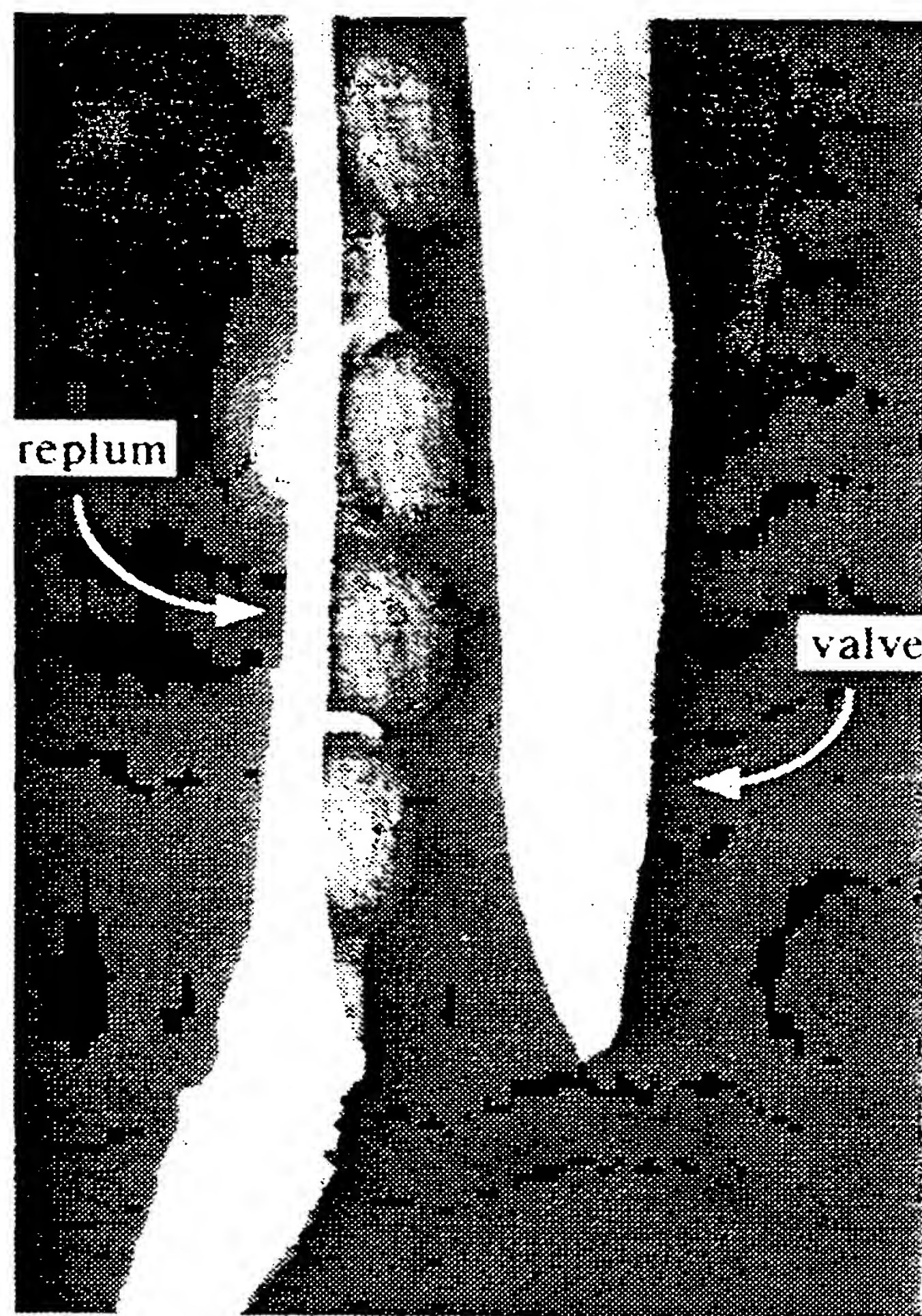


FIG. 2

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WT

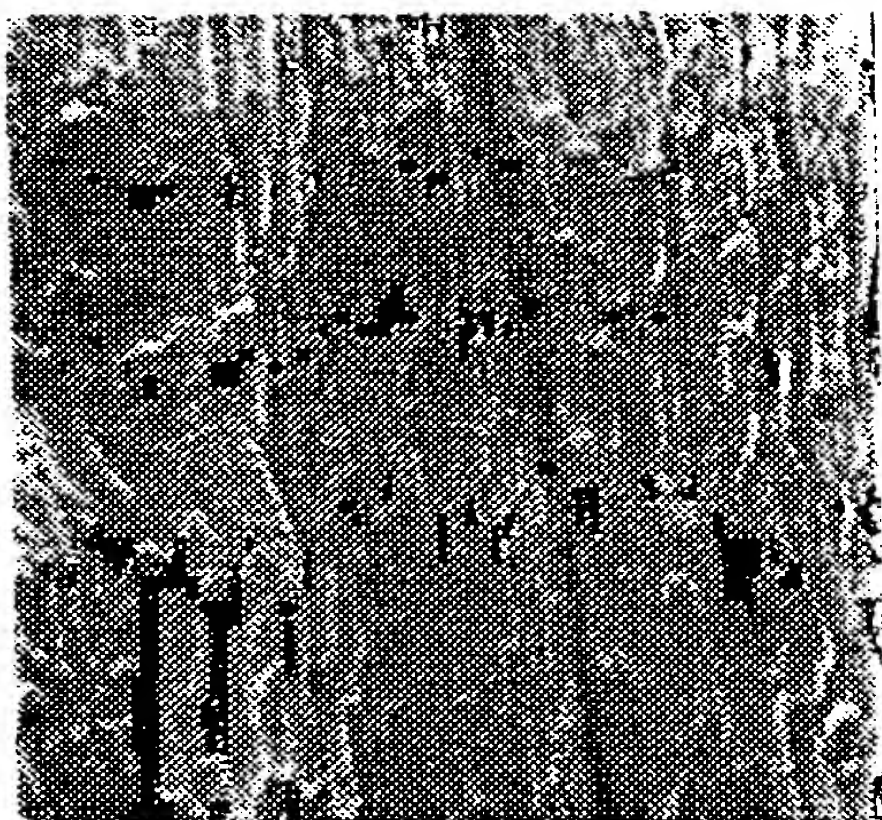


FIG. 3A

35S::AGL8

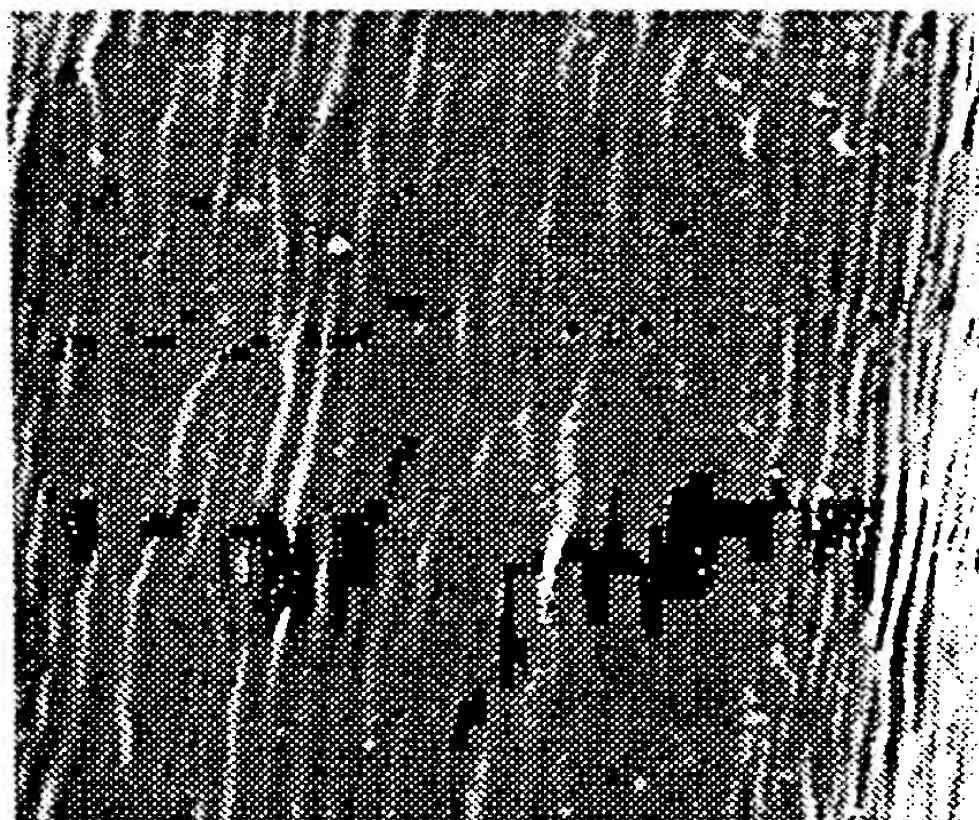


FIG. 3B

SUBSTITUTE SHEET (RULE 26)

# *AGL5* genomic region

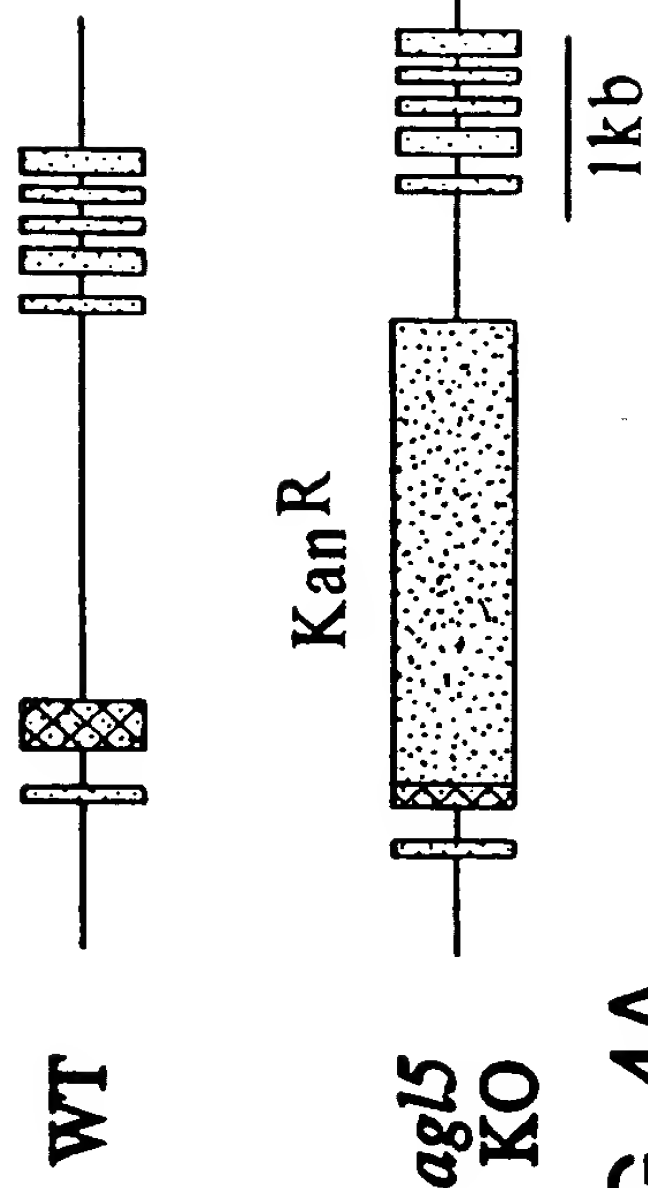


FIG. 4A

# *AGL1* genomic region

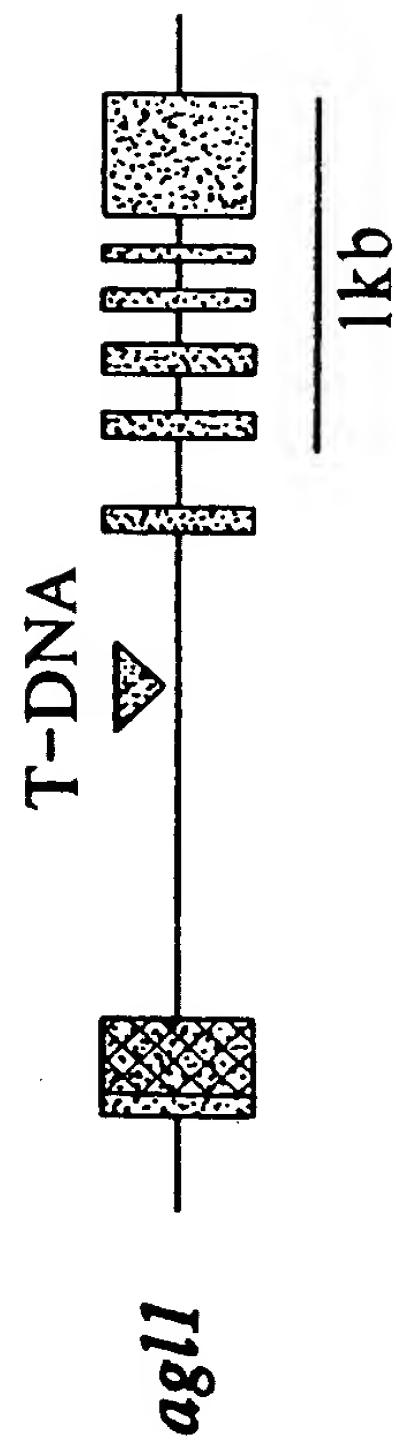
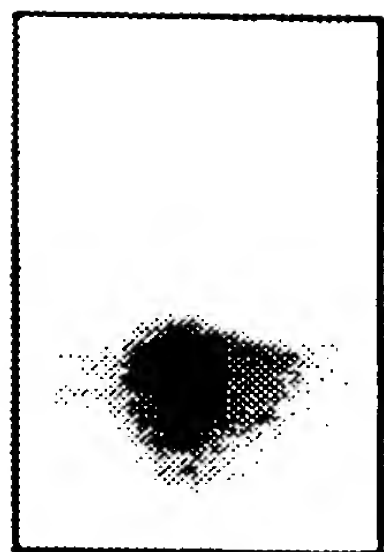


FIG. 4B

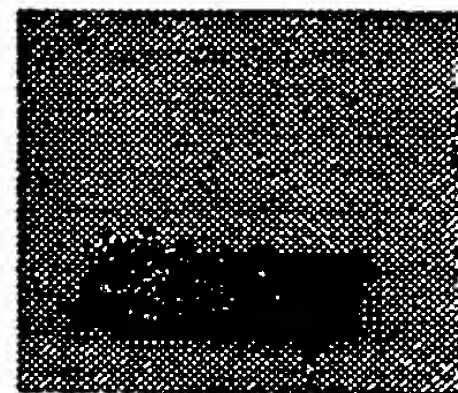
WT *agl5*



*AGL5*  
(3' cDNA)

FIG. 4C

WT *agl1*



*AGL1*  
(3' cDNA)

FIG. 4D



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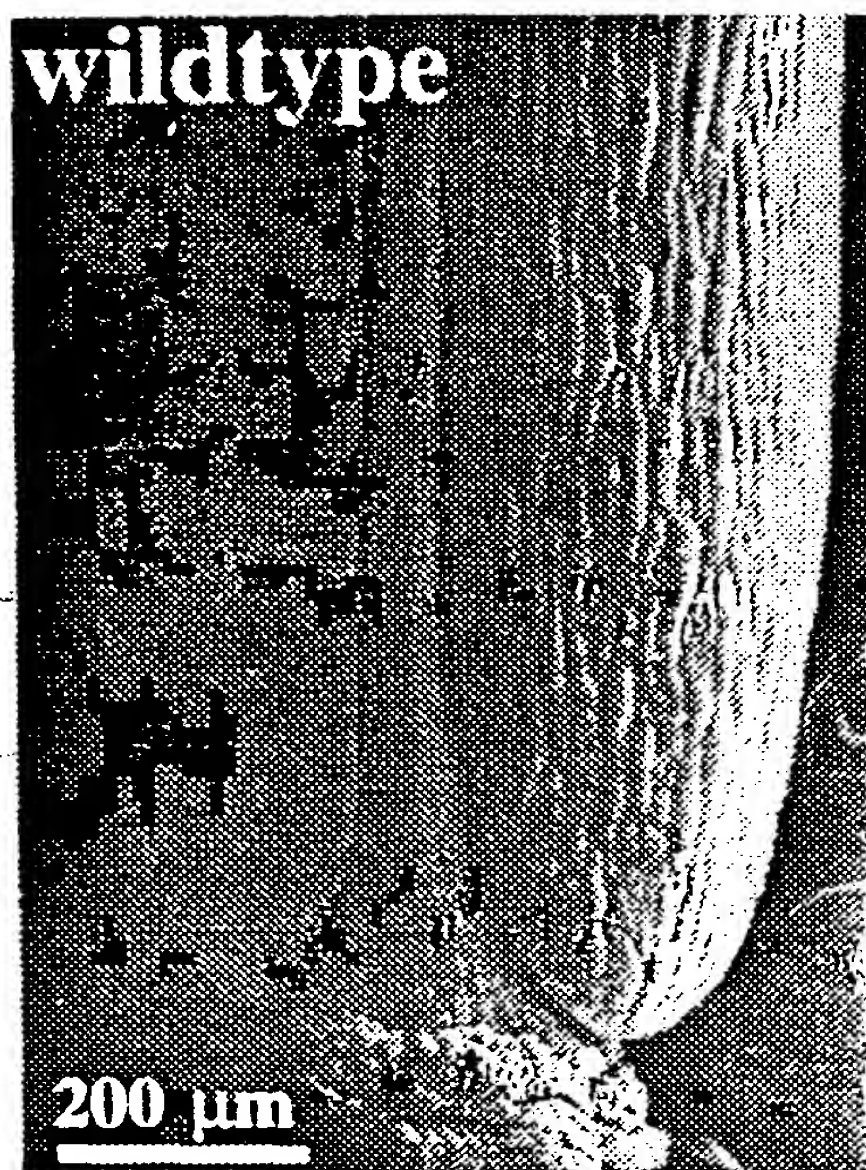


FIG. 5A



FIG. 5B

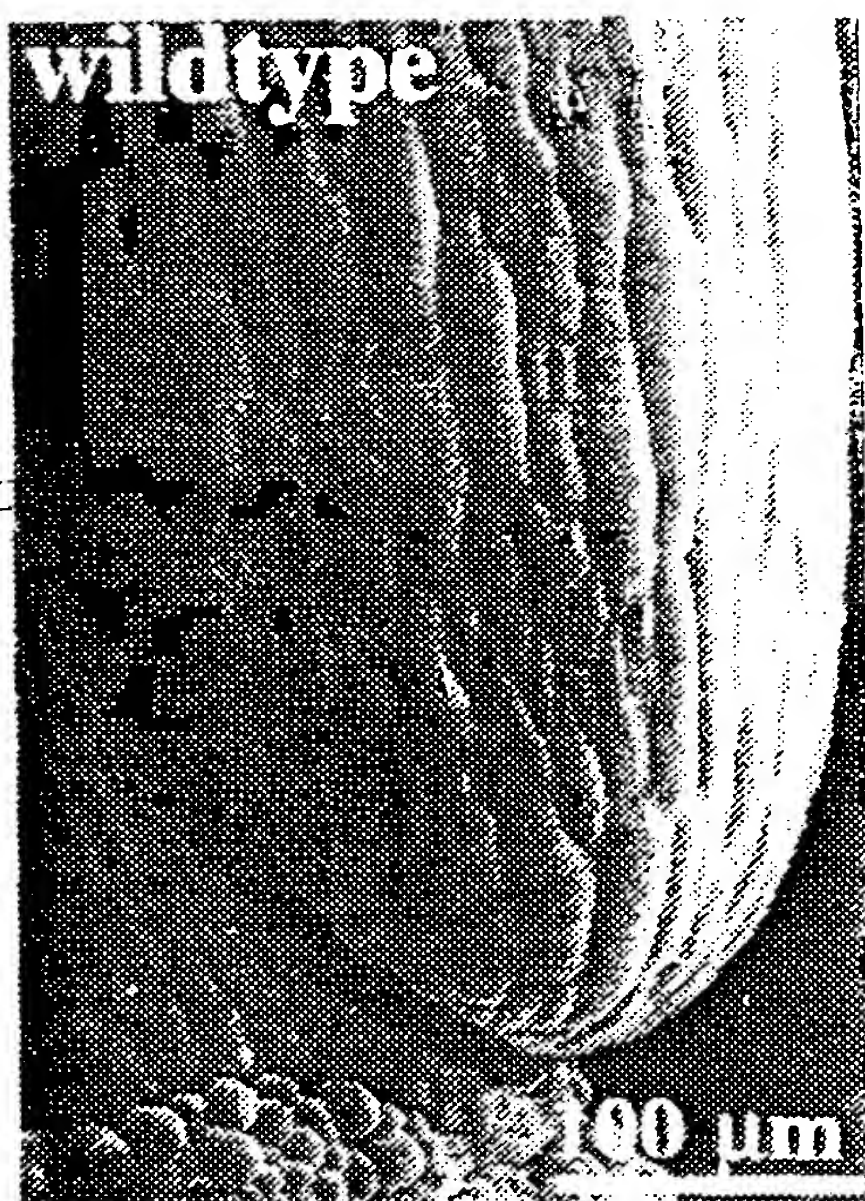


FIG. 5C

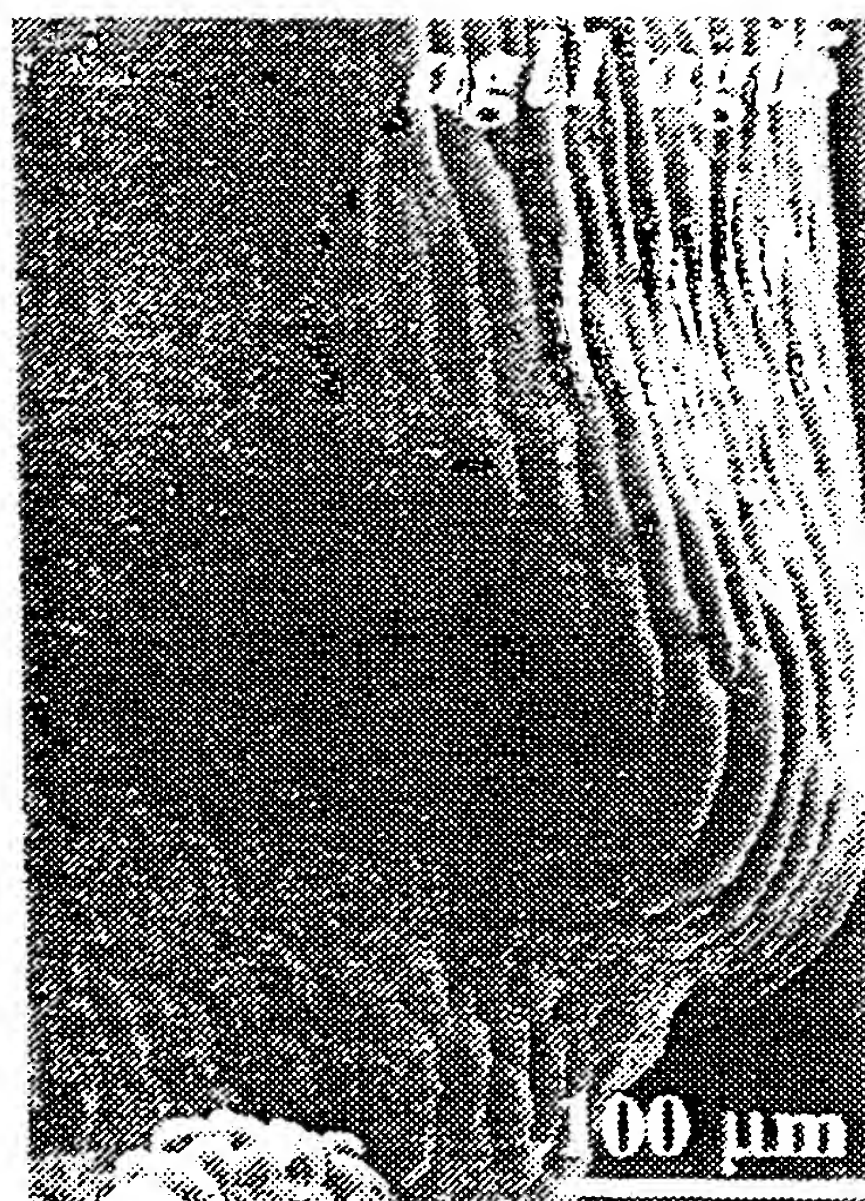


FIG. 5D

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CCCAGAGAGACATAAGAAAGAAAGAGAGAGAGAGATACTT  
TGGTCATTTTCAGGGTTGTCGTTTCTCTCTCTTGTTCCTTGAGATTTTGAAGAGAGAGAGAT  
1 ATGGGAAGAGGTAGGGTTTCAGCTGAAGAGGATAGAGAACAAGATCAATAGGCAAGTTACT  
1 M G R G R V O L K R I E N K I N R O V T  
61 TTCTCAAAGAGAAGGTCTGGTTTGCTCAAGAAAGCTCATGAGATCTCTGTTCTCTGCGAT  
21 F S K R R S G L L K K A H E I S V L C D  
121 GCTGAGGTTGCTCTCATCGTCTTCTCTTCCAAAGGCAAACCTCTTCGAATATTCCACCGAC  
41 A E V A L I V F S S K G K L F E Y S T D  
181 TCTTGCATGGAGAGGATACTTGAACGCTATGATCGCTATTTATATTCAGACAAACAACCTT  
61 S C M E R I L E R Y D R Y L Y S D K Q L  
241 GTTGGCCGAGACGTTTCACAAAGTGAAAATTGGGTTCTAGAACATGCTAAGCTCAAGGCA  
81 V G R D V S Q S E N W V L E H A K L K A  
301 AGAGTTGAGGTACTTGAGAAGAACAAGGAATTTTATGGGGGAAGATCTTGATTCGTTG  
101 R V E V L E K N K R N F M G E D L D S L  
361 AGCTTGAAGGAGCTCCAAAGCTTGGAGCATCAGCTCGATGCAGCTATCAAGAGCATTAGG  
121 S L K E L O S L E H O L D A A I K S I R  
421 TCAAGAAAGAACCAAGCTATGTTCTGAATCCATATCTGCGCTCCAGAAGAAGGATAAAGCC  
141 S R K N O A M F E S I S A L O K K D K A  
481 TTGCAAGATCACAACAATTCGCTTCTCAAAAAGATTAAGGAGAGGGAGAAGAAAACGGGT  
161 L Q D H N N S L L K K I K E R E K K T G  
541 CAGCAAGAAGGACAATTAGTCCAATGCTCCAACCTCTTCTTCAGTTCTTCTGCCTCAATAC  
181 Q Q E G Q L V Q C S N S S S V L L P Q Y  
601 TGCGTAACCTCCTCCAGAGATGGCTTTGTGGAGAGAGTTGGGGGAGAGAACGGTGGTGCA  
201 C V T S S R D G F V E R V G G E N G G A  
661 TCGTCGTTGACGGAACCAAACCTCTCTGCTTCCGGCTTGGATGTTACGTCCTACCACTACG  
221 S S L T E P N S L L P A W M L R P T T T  
721 AACGAGTAGAACTATCTCACTCTTTATAATATAATGATAATATAATTAATGTTTAATATT  
241 N E \*  
781 TTCATAACATTCAGCATTTTTTTTGGTGACTTATACTCATTATTAATACCGATATGTTTTA  
841 GCTAGTCATATTATATGTATGATGGAACCTCCGTTGTGCGAGACGTATGTACGTAAGCTATC  
901 ATTAGATTCACTGCGTCTTAAGAACAAGATTTCATATCTTGGTAATGATTTCTCATGAAA  
961 TAn

FIG. 6

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60  
AGATCTGCAA CAGTGAAAAG AGAAAACAAA ATGGACTTGA AGAGGTTTTG ACAATGCCAG  
120  
AGATAATGCT TATTCCTTAA TATGTTGCCA GCCAAGTGTC AAATTGGCTT TTTAAATATG  
180  
GATTTCTGTA TCAGTGGTCA TATTGTGGA TCCAACGTAT TCATCATCAA GTTCTCAAGT  
240  
TTGCTTTCAG TGCAATTCTA ATTCACACGT TTAACCTTAA CATGCATGTC ATTATAATTA  
300  
CTTCTTCACT AAGACACAAT ACGGCAAACC TTTCAGATTA TATTAATCTC CATAAATGAA  
360  
ATAATTAACC TCATAATCAA GATTCAATGT TTCTAAATAT ATATGGACAA AATTTACACG  
420  
GAAGATTAGA TACGTATATT AGTAGATTTA GTCTTTCGTT TGTGCGATAA GATTAACCAC  
480  
CTCATAGATA GTAATATCAT TGTCAAATTC CTCTCGGTTT AGTCGCTAAA TTGTATCTTT  
540  
TTTAAGCCTA AAAGTAGTGT ATTGCGATAT GACTTATCGT CCTAACTTTT TTTTAAATTA  
600  
ACAAAAAAT CGAAAAGAAA ATAATCTGTT AAATATTTTT TAAGTACTCC ATTAAGTTTA  
660  
GTTTCTATTT AAAAAATGCT TGAAATTTGA CAGTTATGTT CAACAATTTT GAATCATGAG  
720  
CGATGTCTAG ATACTCAGAA TTTAATCAAG ATGTCTTATC AAATTGTGTT TCACTCGAGG  
780  
ACCCACGCAA AAGAAAAGAC TAATATGATT TTTATTTGGT CTGGATATTT TTGTAGAGGA  
840  
TGAAACTAAG AGAGTGAAAG ATTCGAAATC CACAATGTTC AAGAGAGCTC AAAGCAAAAA  
900  
GAAAAATGAA GATGAAGGAC TAAAGAACAA TAAGCAACTA CTTATACCCT ATTTCCATAA

FIG. 7A

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960  
\* \* \* \* \*  
AGGATTCAGG TACTAGGAGA AGTTGAGGCA AGTTNNNNNN NATTGATTCA AATTTTCATT  
1020  
\* \* \* \* \*  
TATTTTACACA ATTTAATTCA CCTAAGTTAT TATGCATTTTC TCATCATTGG TACATTTTCT  
1080  
\* \* \* \* \*  
GTATAGCGTA TTTACATATA TGAAATAAAT TAAATATGTC CTCACGTTGC AAGTAGTTAA  
1140  
\* \* \* \* \*  
TGAATGTCCC CACGCCAAAA AAAATCCCTC CAAATATGTC CACCTTTTCT TTTCTTTTAA  
1200  
\* \* \* \* \*  
ATTCCAAAAT TACCATAAAC TTTTGTTTAA CAAAAGATTT CTAGAAATTG AGGAAGATAT  
1260  
\* \* \* \* \*  
CCTAAATGAT TCATGAATCC TTCAATAATC TGAAGTTTGC GATATTTTCG ATTTTCTTCA  
1320  
\* \* \* \* \*  
AGAGTTGCGA TATTTGTAAT TTGGTGACCT TAAACTTTTT TTGATAAAGA GTAAACGTTT  
1380  
\* \* \* \* \*  
TTTCTTAAAA GTAAAACTTG ATTTTATGTT TTAGGGTTCT AGCTCAACTT TGTATTATAT  
1440  
\* \* \* \* \*  
TTCTTGCAAA AAGAGTTCGT TAACTGCATT CTTCAACACT ATAAAGTGAT TATCAAAAAC  
1500  
\* \* \* \* \*  
ATCTTCATGA ACATTAAGAA AAACAATATT TGGTTTCGGT TAGAGCTTGG TTTTGCTTGG  
1560  
\* \* \* \* \*  
CTTGATTCAC ATACCCATTC TAGACTTTGG CATAAATTG ATACGATAGA GAGTATCTAA  
1620  
\* \* \* \* \*  
TGGTAATGCA GAAGGGTAAA AAAAGGAAGA GAGAAAAGGT GAGAAAGATT ACCAAAAATA  
1680  
\* \* \* \* \*  
AGGAGTTTCA AAAGATGGTT CTGATGAGAA ACAGAGCCCA TCCCTCTCCT TTTCCCCTTC  
1740  
\* \* \* \* \*  
CCATGAAAGA AATCGGATGG TCCTCCTTCA ATGTCCTCCA CCTACTCTTC TCTTCTTTCT  
1800  
\* \* \* \* \*  
TTTTTCTTT CTTATTATTA ACCATTTAAT TAATTTCCCC TTCAATTTC GTTTCTAGTT  
1860  
\* \* \* \* \*

FIG. 7B

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CTGTAAAAAG AAAATACACA TCTCACTTAT AGATATCCAT ATCTATTTAT ATGCATGTAT

1920

AGAGAATAAA AAAGTGTGAG TTTCTAGGTA TGTGAGTAT GTGCTGTTTG GACAATTGTT

1980

AGATGATCTG TCCATTTTTT TCTTTTTTCT TCTGTGTATA AATATATTTG AGCACAAAGA

2040

AAACTAATA ACCTTCTGTT TTCAGCAACT AGGGTCTTAT AACCTTCAAA GAAATATTCC

2100

TTCAATTGAA AACCCATAAA CCAAATAGA TATTACAAA GGAAAGAGAG ATATTTTCAA

2160

GAACAACATA ATTAGAAAAG CAGAAGCAGC AGTTAAGTGG TACTGAGATA AATGATATAG

2220

TTTCTCTTCA AGAACAGTTT CTCATTACCC ACCTTCTCCT TTTTGCTGAT CTATCGTAAT

2280

CTTGAGAACT CAGGTAAGGT TGTGAATATT ATGCACCATT CATTAAACCCT AAAAATAAGA

2340

GATTTAAAAT AAATGTTTCT TCTTTCTCTG ATTCTTGTGT AACCAATTCA TGGGTTTGAT

2400

ATGTTTCTTG GTTATTGCTT ATCAACAAAG AGATTTGATC ATTATAAAGT AGATTAATAA

2460

CTCTTAAACA CACAAAGTTT CTTTATTTTT TAGTTACATC CCTAATTCTA GACCAGAACA

2520

TGGATTGAT CTATTTCTTG GTTATGTATC TTGATCAGGA AAAGGGATTT GATCATCAAG

2580

ATTAGCCTTC TCTCTCTCTC TCTAGATATC TTTCTTGAAT TTAGAAATCT TTATTTAATT

translation  
start

2640

ATTGCGTGAT GTCATATATG GATCAATGGA GGAAGGTGGG AGTAGTCACG ACGCAGAGAG

2700

TAGCAAGAAA CTAGGGAGAG GGAAATAGA GATAAAGAGG ATAGAGAACA CAACAAATCG exon 1

2760

TCAAGTTACT TTCTGCAAAC GACGCAATGG TCTTCTCAAG AAAGCTTATG AACTCTCTGT

FIG. 7C

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2820

CTTGTGTGAT GCCGAAGTTG CCTTCGTCAT CTTCTCCACT CGTGGCCGTC TCTATGAGTA

2880

CGCCAACAAC AGGTACGCTT CTCCTACTCT ATTTCTTGAT CTGTTTTTCT TAATTTTAAC

2940

TAAACAAGAT CCTAGTTCAA ATGATAACAA AGTGGGGATT GAGAGCCAAG ATTAGGGTTT

3000

GGTTAATTTA GAAAACCAGA TTCACTTGT TGATACATTT AATATCTCTC TAGCTAGATT

3060

TAGTACTCTC TCCTCTATAT ATGTGTGGGT GTGTGTGTAA GTGTGTATAT GTATGCAAAT

3120

GCAAGAAGAA GAAGAAAAG TTATCTTGTC TTCTCAAATT CTGATCAGCT TTGACCTTAG

3180

TTCACTCTT TTTTCTGCAA ATCATTGAA CCTGATGCAT GTCAGTTTCT ACAATACT

3240

TTTAATTTTG ACGGCCCATC AAATTCCTA GGGTTTACTT CAGTGAACAA AATTGGGTTC

3300

TTGACACGAT TTAGCATGTA TATATAAAAA TAGGGGATGA TCAAGACTTA TGTAACCTCT

3360

GTCTGGTGAA ACTAGGGACA AAGTCTACTG ATGAGTTGTC ACTAGGGATC CATTGATCA

3420

TTTAATCCCA ACAAAAATGA AACAAAATTT TGAGAATTTA TATGCTGAAG TTTTCAACC

3480

CTCTTTTTTA AATAACTTTA TATTATGTAG ATTTGTATTT AGGGTAATTT GTCCAACCTAG

3540

AAGTCCTAAA AATCAATAAA CACACGGATG ACTTTGTCTA ACATTGTATC AGTCATCAAA

3600

TGTAATAATTG TACAAATAAT GAAATTAAAG ATTTAGTCTC TTTTATTTTT TTGTTTAGG

3660

GTGTATATAT ATATATATAT GTATATTTGT TGCATTGATA TATCAATGAG AGGGAGAGAA

3720

FIG. 7D

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CTCAGAGAAG TGTCGGAAAT TAAAATGGTA CGAGCCAATT GGAATCTCTG GCATTCTGAG  
3780  
CTTCATTTGT TTGTTATTAG AAAAAAAAAA AAAAAATCCT TTAAAGATAC CTTCATGATG  
3840  
ACATTGAATC ATGTAATATA CACGATACAT GGTCTAATTC CTCCTCAAAC CCTAATTACC  
3900  
AATTTGAAA CCATAATATT TACTAGTATG TTTATATATC CTTACTTTAA GACATTGTTT  
3960  
GTTTATAATA CCTTGTGAAT TAAGAAAAAA AAAAAAAAC TTGTGGATCT ATTCAAGCCA  
4020  
TGTGTTAGAA TAAATTTATA AATTTTCTCC TCGTACTGGT CAGATATTGG TCCAAACTCC  
4080  
AAAGCCTTCC CTTTTCAGGA AAAAAACAT TTCGAAATTA ACTCTAATTA ATCAAGAATT  
4140  
TCCTACAATG TATACATCTA ATGTTTTTTC CGCGATCTTA CTTATTAGTG TGAGGGGTAC  
4200  
AATTGAAAGG TACAAGAAAG CTTGTTCCGA TGCCGTCAAC CCTCCTTCCG TCACCGAAGC exon 2  
4260  
TAATACTCAG GTACCAATTT ATATTGTTTG ATTCTCTTTG TTTTATCTTC TTCTTTTCAT  
4320  
TATATATATG ATCAACAAAA AATATAACCT AAAAAAGAG AGAGTTCAAG GAAATGCATT  
4380  
GAAACGGTTT CGTTATGGTG TTTGAATACA TGGATTTTTG AAGTACTATC AGCAAGAAGC  
4440 exon 3  
CTCTAAGCTT CGGAGGCAGA TTCGAGATAT TCAGAATTCA AATAGGTAAT TCATTAACCT  
4500  
TTCATGAACT CTTGATTTG GTATTAGGTC ACTTAATTTG GTGTCGGTCC AAAAGTCCGC  
4560  
TTGTAGTTTT CTTTAGAAGT TGTTTTGTTT AATGTTTCATG TTTACAAATT GAAGGCATAT  
4620 exon 4  
TGTGGGGAA TCACTTGGTT CCTTGAACCT CAAGGAACTC AAAACCTAG AAGGACGTCT

FIG. 7E

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4680  
\* \* \* \* \*  
TGAAAAAGGA ATCAGCCGTG TCCGCTCCAA AAAGTAAAA TCTACGTTGC TCTCTCTCTG

4740  
\* \* \* \* \*  
TGCTCTGTG TCTCTCTCTA TATATAGTCC CTTAGTTTAT ATAGTTCATC ACCCTTTTGT

4800  
\* \* \* \* \*  
GAGAATTTTG CAGATGAGC TGTTAGTGGC AGAGATAGAG TATATGCAGA AGAGGTAAG exon 5

4860  
\* \* \* \* \*  
AACGTTTCTC CCATTCCAAG TAATTAGATC TTTCTTCGTC TTGTGAGGG TTGAGTTT

4920  
\* \* \* \* \*  
CCCATAAATC ATGTGTAGGA AATGGAGTTG CAACACAATA ACATGTACCT GCGAGCAAAG exon 6

4980  
\* \* \* \* \*  
GTTAGCCACG TTCTGTTCCA AATCTTAATC TCAATATCTA CTCTTTTCTT CATTGTATAA

5040  
\* \* \* \* \*  
CTAAGATAAC GTGAATAACA AGAAAACTTT TGTTTTTGGG TTTAATAGAT AGCCGAAGGC

5100  
\* \* \* \* \*  
GCCAGATTGA ATCCGGACCA GCAGGAATCG AGTGTGATAC AAGGGACGAC AGTTTACGAA

5160  
\* \* \* \* \*  
TCCGGTGTAT CTTCTCATGA CCAGTCGCAG CATTATAATC GGAACATAT TCCGGTGAAC

5220 stop  
\* \* \* \* \* codon  
CTTCTTGAAC CGAATCAGCA ATTCTCCGGC CAAGACCAAC CTCCTCTTCA ACTTGTGTAA

5280  
\* \* \* \* \*  
CTCAAACAT GATAACTTGT TTCTTCCCCT CATAACGATT AAGAGAGAGA CGAGAGAGTT

5340  
\* \* \* \* \*  
CATTTTATAT TTATAACGCG ACTGTGTATT CATAGTTTAG GTTCTAATAA TGATAATAAC

5400  
\* \* \* \* \*  
AAACTGTTG TTTCTTTGCT TAATTACATC AACATTTAAA TCCAAAGTTC TAAAACACGT

5460  
\* \* \* \* \*  
CGAGATCCAA AGTTTGTGTCAT ACAAGATTAG ACGCATACAC GATCAGTTAA TAGATTTTAA

5520  
\* \* \* \* \*  
GTGCCTTTTA ATATTTACAT ATAGTTGCAG CTTCGATTAG ATCATGTCCA CCAAACACTC

5580  
\* \* \* \* \*

FIG. 7F

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ACAATTAGAG ACAAGCAAAA CTATAACAT TGATCATAAA ATGATTACAA CATGTCCATA  
\* \* \* \*  
AATTAATTAT GGATTACAAA AATAAAACT TACAAAAGAT CT

FIG. 7G

Sequence Range: 1 to 6138

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|            |            |            |            |            |            |
|------------|------------|------------|------------|------------|------------|
| 10<br>*    | 20<br>*    | 30<br>*    | 40<br>*    | 50<br>*    | 60<br>*    |
| GAATTCGTAA | CAGAATTTAG | TGAATAATAT | TGTAATTACC | AGGCAAGGAC | TCTCCAAACG |
| 70<br>*    | 80<br>*    | 90<br>*    | 100<br>*   | 110<br>*   | 120<br>*   |
| GATAGCTCGA | ATATCGTTAT | TAAAGAGTAA | ATGATCCAAT | ATGTAAGCCA | TTGTTGATCA |
| 130<br>*   | 140<br>*   | 150<br>*   | 160<br>*   | 170<br>*   | 180<br>*   |
| TCTAACATTG | TTGGACTCTC | TATTGCTCGA | AATGATGCAT | ACCTAATCAT | TTATTCAGTT |
| 190<br>*   | 200<br>*   | 210<br>*   | 220<br>*   | 230<br>*   | 240<br>*   |
| AACTATCAAG | TTGCATTTGT | AAAAACCAAA | CATTTAAATT | CAGATTTGAT | ATCACTTACA |
| 250<br>*   | 260<br>*   | 270<br>*   | 280<br>*   | 290<br>*   | 300<br>*   |
| GAGGATAGAG | AAGCATGACT | CCAGGCCTGC | ATGCAACAAG | AAAAAGGAAG | AAAATAATGT |
| 310<br>*   | 320<br>*   | 330<br>*   | 340<br>*   | 350<br>*   | 360<br>*   |
| TAAAAATTTG | ACAAATATAG | TGTTTATTTT | TATTATATGA | GACAGAATTT | GAATAAAATC |
| 370<br>*   | 380<br>*   | 390<br>*   | 400<br>*   | 410<br>*   | 420<br>*   |
| CTACCCAACT | AGAGCATCAA | AACGTTTTGC | AATCGCAATA | ATGAAACCCA | TTTTCTTTTT |
| 430<br>*   | 440<br>*   | 450<br>*   | 460<br>*   | 470<br>*   | 480<br>*   |
| GAGTTTTTAC | TCTTCTTTCA | ACAGAACTT  | TCTCAAACGT | CTTTAGCACT | GTGACGTTAG |
| 490<br>*   | 500<br>*   | 510<br>*   | 520<br>*   | 530<br>*   | 540<br>*   |
| ATATATACAC | AAAAGCTTGA | AATTTCTTCA | AGCAAAAGAA | TCTTTGTGGG | AGTTAAGGCA |
| 550<br>*   | 560<br>*   | 570<br>*   | 580<br>*   | 590<br>*   | 600<br>*   |
| ACAAGCCAGG | TAAAGAATCT | CCAACGCATT | GTTACGTTTT | CATGAACCTA | TTTATTATAT |
| 610<br>*   | 620<br>*   | 630<br>*   | 640<br>*   | 650<br>*   | 660<br>*   |
| GTTCTAAGAA | AGAAAAAAT  | ATCTCAAAGT | AAACGTTGGA | AATTTTCTGA | TGAAGGGAAA |
| 670<br>*   | 680<br>*   | 690<br>*   | 700<br>*   | 710<br>*   | 720<br>*   |
| TCCAAAGTCT | TGGGTTTAGT | ATCCCTATGA | ATGGTATTTG | GAATATGTTT | TCGTCAAAAC |
| 730<br>*   | 740<br>*   | 750<br>*   | 760<br>*   | 770<br>*   | 780<br>*   |
| AAAAGATTCT | TTTCTTTTTC | ACAAGAGTTA | GTGATCAATA | ACTTATGCAC | TAATTAATGA |
| 790<br>*   | 800<br>*   | 810<br>*   | 820<br>*   | 830<br>*   | 840<br>*   |
| GATTGGACGT | ATACACAATT | TGATTATGAT | ACTTGAGTAA | AAATCACCTG | TCCTTTAATT |
| 850<br>*   | 860<br>*   | 870<br>*   | 880<br>*   | 890<br>*   | 900<br>*   |
| TGGAAATCTC | TCTTTCTTAC | CCATTTATAT | ACTACTTCTT | TTCATTAAAA | TTAAATTTCA |

FIG. 8A

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|            |            |            |            |             |            |
|------------|------------|------------|------------|-------------|------------|
| 910        | 920        | 930        | 940        | 950         | 960        |
| *          | *          | *          | *          | *           | *          |
| ATTATCAATC | ATCGTTCAAT | TTGATAAAGA | TTTAACATTT | TTTGTCACAG  | GGCTAGTAAA |
| 970        | 980        | 990        | 1000       | 1010        | 1020       |
| *          | *          | *          | *          | *           | *          |
| AGCAATCTTT | ACATAATTCA | TCTTTCTTAC | ATATATATAT | TACCTTTTTTC | TTCATTAGTA |
| 1030       | 1040       | 1050       | 1060       | 1070        | 1080       |
| *          | *          | *          | *          | *           | *          |
| TTCTATTTGA | TTATGATTAT | TTTGTCATAA | AGCTAGTAAA | TTAAACACTC  | GATATGAGAA |
| 1090       | 1100       | 1110       | 1120       | 1130        | 1140       |
| *          | *          | *          | *          | *           | *          |
| TTATATTACT | TCACGCTAAT | TAACTCTTAA | CACAACAAGA | ACTAGTGCAT  | ATTCAACTTT |
| 1150       | 1160       | 1170       | 1180       | 1190        | 1200       |
| *          | *          | *          | *          | *           | *          |
| CAAAGCATAT | ACTATATATT | GAGAATATAG | ACCACGAAAG | TCAATCAAAA  | GACCTACCAG |
| 1210       | 1220       | 1230       | 1240       | 1250        | 1260       |
| *          | *          | *          | *          | *           | *          |
| CTCTCATCAA | GTTCTTTCTT | GAAATGATTT | TGCAGAATTT | CCAACTTAAT  | TAATTCGACA |
| 1270       | 1280       | 1290       | 1300       | 1310        | 1320       |
| *          | *          | *          | *          | *           | *          |
| TGAATGTGAA | AATGTGTGTT | GCTCGTTAAG | AAAATTGAAT | AGAAGTACAA  | TGAAAATGAT |
| 1330       | 1340       | 1350       | 1360       | 1370        | 1380       |
| *          | *          | *          | *          | *           | *          |
| GAGGAATGGG | CAAAACACAA | AAGAGTTTCC | TTTCGTAACT | ACAATTAATT  | AATGCAAATC |
| 1390       | 1400       | 1410       | 1420       | 1430        | 1440       |
| *          | *          | *          | *          | *           | *          |
| TGAGAAAGGG | TTCATGGATA | ATGACTACAC | ACATGATTAG | TCATTCCCCG  | TGGGCTCTCT |
| 1450       | 1460       | 1470       | 1480       | 1490        | 1500       |
| *          | *          | *          | *          | *           | *          |
| GCTTTCATTT | ACTTTATTAG | TTTCATCTTC | TCTAATTATA | TTGTTCGCATA | TATGATGCAG |
| 1510       | 1520       | 1530       | 1540       | 1550        | 1560       |
| *          | *          | *          | *          | *           | *          |
| TTCTTTTGTC | TAAATTACGT | AATATGATGT | AATTAATTAT | CAAAATAAAT  | ATTCAAATTG |
| 1570       | 1580       | 1590       | 1600       | 1610        | 1620       |
| *          | *          | *          | *          | *           | *          |
| CCGTTGGACT | AACCTAATGT | CCAAGATTAA | GACTTGAACA | TAAGAATTTT  | GGAAAAACTA |
| 1630       | 1640       | 1650       | 1660       | 1670        | 1680       |
| *          | *          | *          | *          | *           | *          |
| AACCAGTTAT | AATATATACT | CTTAAATTGC | CATTTCTGAA | CACAACCAAA  | TAATAATATA |
| 1690       | 1700       | 1710       | 1720       | 1730        | 1740       |
| *          | *          | *          | *          | *           | *          |
| TACTATTTAC | AGTTTTTTTT | AATTGGCAAG | AACACTGAAA | TCTTATTCAT  | TGTCTCGCTT |
| 1750       | 1760       | 1770       | 1780       | 1790        | 1800       |
| *          | *          | *          | *          | *           | *          |
| GGTAGTTGAC | AAGTTATAAC | ACTCATATTC | ATATAACCCC | ATTCTAACGT  | TGACGACGAA |
| 1810       | 1820       | 1830       | 1840       | 1850        | 1860       |
| *          | *          | *          | *          | *           | *          |

FIG. 8B

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|                   |            |            |            |            |            |
|-------------------|------------|------------|------------|------------|------------|
| CACTCATATA        | AACCACCCAA | ATTCTTAGCA | TATTAGCTAA | ATATTGGTTT | AATTGGAAAT |
| 1870              | 1880       | 1890       | 1900       | 1910       | 1920       |
| *                 | *          | *          | *          | *          | *          |
| ATTTTTTTTA        | TATATAAAAT | GCCAGGTAAA | TATTAACGAC | ATGCAATGTA | TATAGGAGTA |
| 1930              | 1940       | 1950       | 1960       | 1970       | 1980       |
| *                 | *          | *          | *          | *          | *          |
| GGGCAATAAA        | AAGAAAAGGA | GAATAAAAAG | GGATTACCAA | AAAAGGAAAG | TTTCCAAAAG |
| 1990              | 2000       | 2010       | 2020       | 2030       | 2040       |
| *                 | *          | *          | *          | *          | *          |
| GTGATTCTGA        | TGAGAAACAG | AGCCCATACC | TCTCTTTTTT | CCTCTAAACA | TGAAAGAAAA |
| 2050              | 2060       | 2070       | 2080       | 2090       | 2100       |
| *                 | *          | *          | *          | *          | *          |
| ATTGGATGGT        | CCTCCTTCAA | TGCTCTCTCC | CCACCCAATC | CAAACCCAAC | TGTCTTCTTT |
| 2110              | 2120       | 2130       | 2140       | 2150       | 2160       |
| *                 | *          | *          | *          | *          | *          |
| CTTCTTTTTT        | TCTTCTTTCT | AATTTGATAT | TTTCTACCAC | TTAATTCCAA | TCAATTTCAA |
| 2170              | 2180       | 2190       | 2200       | 2210       | 2220       |
| *                 | *          | *          | *          | *          | *          |
| ATTTCAATCT        | AAATGTATGC | ATATAGAATT | TAATTAAAAG | AATTAGGTGT | GTGATATTTG |
| 2230              | 2240       | 2250       | 2260       | 2270       | 2280       |
| *                 | *          | *          | *          | *          | *          |
| AGAAAATGTT        | AGAAGTAATG | GTCCATGTTC | TTTCTTTCTT | TTTCCTTCTA | TAACACTTCA |
| 2290              | 2300       | 2310       | 2320       | 2330       | 2340       |
| *                 | *          | *          | *          | *          | *          |
| GTTTGAAAAA        | AAACTACCAA | ACCTTCTGTT | TTCTGCAAAT | GGGTTTTTAA | ATACTTCCAA |
| 2350              | 2360       | 2370       | 2380       | 2390       | 2400       |
| *                 | *          | *          | *          | *          | *          |
| AGAAATATTC        | CTCTAAAAGA | AATTATAAAC | CAAAACAGAA | ACCAAAAACA | AAAAATAAAG |
| 2410              | 2420       | 2430       | 2440       | 2450       | 2460       |
| *                 | *          | *          | *          | *          | *          |
| TTGAAGCAGC        | AGTTAAGTGG | TACTGAGATA | ATAAGAATAG | TATCTTTAGG | CCAATGAACA |
| 2470              | 2480       | 2490       | 2500       | 2510       | 2520       |
| *                 | *          | *          | *          | *          | *          |
| AATTAACTCT        | CTCATAATTC | ATCTTCCCAT | CCTCACTTCT | CTTCTTTTCT | GATATAATTA |
| 2530              | 2540       | 2550       | 2560       | 2570       | 2580       |
| *                 | *          | *          | *          | *          | *          |
| ATCTTGCTAA        | GCCAGGTATG | GTTATTGATG | ATTTACACTT | TTTTTTAAAA | GTTTCTTCCT |
| 2590              | 2600       | 2610       | 2620       | 2630       | 2640       |
| *                 | *          | *          | *          | *          | *          |
| TTTCTCCAAT        | CAAATTCCTC | AGTTAATCCT | TATAAACCAT | TTCTTTAATC | CAAGGTGTTT |
| 2650              | 2660       | 2670       | 2680       | 2690       | 2700       |
| *                 | *          | *          | *          | *          | *          |
| GAGTGCAAAA        | GGATTTGATC | TATTTCTCTT | GTGTTTATAC | TTCAGCTAGG | GCTTATAGAA |
| translation start | 2710       | 2720       | 2730       | 2740       | 2750       |
| *                 | *          | *          | *          | *          | *          |
| ATG               | GAGGGTG    | GTGCGAGTAA | TGAAGTAGCA | GAGAGCAGCA | AGAAGATAGG |
|                   |            |            |            |            | GAGAGGGAAG |
|                   |            |            |            |            | 2760       |
|                   |            |            |            |            | *          |
|                   |            |            |            |            | exon 2     |

FIG. 8C

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|            |            |            |            |            |             |
|------------|------------|------------|------------|------------|-------------|
| 2770       | 2780       | 2790       | 2800       | 2810       | 2820        |
| *          | *          | *          | *          | *          | *           |
| ATAGAGATAA | AGAGGATAGA | GAACACTACG | AATCGTCAAG | TCACCTTCTG | CAAACGACGC  |
| 2830       | 2840       | 2850       | 2860       | 2870       | 2880        |
| *          | *          | *          | *          | *          | *           |
| AATGGTTTAC | TCAAGAAAGC | TTATGAGCTC | TCTGTCTTGT | GTGACGCTGA | GGTTGCTCTT  |
| 2890       | 2900       | 2910       | 2920       | 2930       | 2940        |
| *          | *          | *          | *          | *          | *           |
| GTCATCTTCT | CCACTCGAGG | CCGTCTCTAC | GAGTACGCCA | ACAACAGGTA | CACATCTTTT  |
| 2950       | 2960       | 2970       | 2980       | 2990       | 3000        |
| *          | *          | *          | *          | *          | *           |
| AGCTAGATCT | TGATTTTGTT | GAATTTTTTT | TCTAGAATAA | AGTTTCGACT | CTTCTGGTGG  |
| 3010       | 3020       | 3030       | 3040       | 3050       | 3060        |
| *          | *          | *          | *          | *          | *           |
| GTTTTTCAAT | CTTTATGGTC | TCTTTATAGT | TTTTTTCCTT | AGTTTCTCTG | AAGCTCAAAT  |
| 3070       | 3080       | 3090       | 3100       | 3110       | 3120        |
| *          | *          | *          | *          | *          | *           |
| CTCTTTAAAA | ATCCCCAAAA | TTAGGGTTTG | TTTAAAACTA | GGAACCCTA  | CTTTAACTTC  |
| 3130       | 3140       | 3150       | 3160       | 3170       | 3180        |
| *          | *          | *          | *          | *          | *           |
| TTTCTCTTAG | TAAAAAAGCA | GTGAGGGTCT | TCTCTGATCA | TTAATTAGCA | TCCCCCATACT |
| 3190       | 3200       | 3210       | 3220       | 3230       | 3240        |
| *          | *          | *          | *          | *          | *           |
| CTTGTTCCAG | TCACCTTTTC | TCCACAAATC | CTTATAACAG | TATCTATATA | TGTATCTATT  |
| 3250       | 3260       | 3270       | 3280       | 3290       | 3300        |
| *          | *          | *          | *          | *          | *           |
| TATGTCAGTT | TGTACAAGAC | ACTTCGATCA | ATTGATGAC  | CCATCAAGTT | TTATTTCTGC  |
| 3310       | 3320       | 3330       | 3340       | 3350       | 3360        |
| *          | *          | *          | *          | *          | *           |
| AGATTGATCA | TTAGGTTTCC | ATCATAGTAA | TGAAAAAGTA | GGGTTCTTGA | TAAAATTATA  |
| 3370       | 3380       | 3390       | 3400       | 3410       | 3420        |
| *          | *          | *          | *          | *          | *           |
| ATAATATATA | TTATTTGGCT | ATATAAAAAA | GCTATGTAGA | TTCCTTAAAA | ATTGATTAC   |
| 3430       | 3440       | 3450       | 3460       | 3470       | 3480        |
| *          | *          | *          | *          | *          | *           |
| TAGGGAGAGA | CTAGTAGGTG | TTTGTCTTCT | GACACTTCTC | TAATCTTTTG | GTGAATCCTT  |
| 3490       | 3500       | 3510       | 3520       | 3530       | 3540        |
| *          | *          | *          | *          | *          | *           |
| TTGTTAAATC | AAGAAAATGA | ATCAGGGACA | AAGCTTATTG | TTGAGTCACT | TAATTAATCA  |
| 3550       | 3560       | 3570       | 3580       | 3590       | 3600        |
| *          | *          | *          | *          | *          | *           |
| TCCGATCCAT | CAATCAAGAA | AAATAACGAA | ACAGAAAATT | TTGATTTTGG | ATTGTTATTT  |
| 3610       | 3620       | 3630       | 3640       | 3650       | 3660        |
| *          | *          | *          | *          | *          | *           |
| TCTCCACTTC | AAGTTGGGGA | CTTGTCATTT | CCGTTTTTCT | ATACGTTTCC | AGCTATTAAAC |
| 3670       | 3680       | 3690       | 3700       | 3710       | 3720        |
| *          | *          | *          | *          | *          | *           |

FIG. 8D

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AGCTCATGTT CATTTACCA TTTTGATTAT TTGCTGCTT TTTAAAGATA AATGTTTTCA  
3730 3740 3750 3760 3770 3780  
\* \* \* \* \*  
AAAATATTGT TTTATTTGC TTGGCTAGTT AATACTATAA TTGAGGTTGA TGTATGACTA  
3790 3800 3810 3820 3830 3840  
\* \* \* \* \*  
TAATCTATAA GTCAAGTCTC ATATCATGGA TCTAAGTTAA AACTAGTAAA TTTGTAGTTT  
3850 3860 3870 3880 3890 3900  
\* \* \* \* \*  
CAATGTGAAC TTTCACAACG ACTAAAGAAC TGATCTGAAG TTTATAATGG ACATGACTAA  
3910 3920 3930 3940 3950 3960  
\* \* \* \* \*  
TTTGATTAAAC AAAAGAGGAA TGCATTATGT ATGTAGAAAC ATGTGATATA TATATGTTTC  
3970 3980 3990 4000 4010 4020  
\* \* \* \* \*  
TATTATCAAA AGTGTAAGTTA ACTTTCTTAT TTCAAACACC CTCATGCTTT AGTAGTATCT  
4030 4040 4050 4060 4070 4080  
\* \* \* \* \*  
TACTTTTGAC ATTTCTCAAC TTCAGCTTTC CATTATACAA CAGCACAATG TAAATTACTT  
4090 4100 4110 4120 4130 4140  
\* \* \* \* \*  
GTATATGAAT ATGAAAGCAT AACGTTATGC AAAGATTTCT AGCTTTTCTT TTTCTGTTTT  
4150 4160 4170 4180 4190 4200  
\* \* \* \* \*  
GCAAAAGATT TACAAATATC ATGTTCTTGG TAAAAACATA CTTGCCTCAG CCACATATGC  
4210 4220 4230 4240 4250 4260  
\* \* \* \* \*  
ATGTAAATGT AATGTTCAAA TATTAATTCA GGAAAAACAA AGAAGAAGCA AAATTAGCTT  
4270 4280 4290 4300 4310 4320  
\* \* \* \* \*  
CTAGAGTAGG GAATCTATTG ACTTGACCTG AAAATCACTT CTTTTTCTTA AAGCCTAGTA  
4330 4340 4350 4360 4370 4380  
\* \* \* \* \*  
GTGAATTTTT TAATCTAATT AGGCCAAAAT ATATACTAGC CTAAAATATA ATTGGATTT  
4390 4400 4410 4420 4430 4440  
\* \* \* \* \*  
TGTGTCGTAC ATAAATGGG ACCAATTCCA ATTAATAAG AGCATATGCA ATTCAAATTC  
4450 4460 4470 4480 4490 4500  
\* \* \* \* \*  
TTTTTATTTT CTTCCTCCGAT TTGCTACTTC TTCTTTTGT ATGTTTCAA ATTAGGATTA  
4510 4520 4530 4540 4550 4560  
\* \* \* \* \*  
CACTTTTTTG GGGAAGTACA CATTAGGGTC TTCTCGAACT TTGATTATAC ATATATATAT  
4570 4580 4590 4600 4610 4620  
\* \* \* \* \*  
ATATATATAT ATATAACTTT GTGAGATGTC ACTGTTAATA GATAATAGGC AATAACAATA

FIG. 8E

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|            |            |             |            |            |            |
|------------|------------|-------------|------------|------------|------------|
| 4630       | 4640       | 4650        | 4660       | 4670       | 4680       |
| *          | *          | *           | *          | *          | *          |
| ATATCCAAAA | AAGAAGGCGC | AAACAAATCA  | TATACTATAT | GGTACTGGTC | CATTCACTAT |
| 4690       | 4700       | 4710        | 4720       | 4730       | 4740       |
| *          | *          | *           | *          | *          | *          |
| TTTGTCGGTT | GAATTTAAGG | TTTGCGGTAC  | AAACTTTGTT | TCAAACCTTT | ATTATTCCGT |
| 4750       | 4760       | 4770        | 4780       | 4790       | 4800       |
| *          | *          | *           | *          | *          | *          |
| CTTCTGTGT  | GTTTTGTATA | TCCAGAAGAT  | AAAAATATCA | ATTCTTTTAA | CGACTTCATA |
| 4810       | 4820       | 4830        | 4840       | 4850       | 4860       |
| *          | *          | *           | *          | *          | *          |
| TATATATATA | TATATATATA | TATATATATT  | TTTCTCTTCT | GGTTTTAGTG | TTTGAATCCA |
| 4870       | 4880       | 4890        | 4900       | 4910       | 4920       |
| *          | *          | *           | *          | *          | *          |
| ACAGTTATAG | TTTCGTGTGT | CTTGTMTTAA  | CTTGTGGTGG | TTTAAGTTTG | AGATTTTCAC |
| 4930       | 4940       | 4950        | 4960       | 4970       | 4980       |
| *          | *          | *           | *          | *          | *          |
| CGATTGCATC | TATTTACATA | TATAGCTACC  | ACAAAAAAGA | TTGCATTTTA | AAATCTTTTC |
| 4990       | 5000       | 5010        | 5020       | 5030       | 5040       |
| *          | *          | *           | *          | *          | *          |
| CTTGTGTGA  | ATGTTGATGA | AGTGTGAGAG  | GAACAATAGA | AAGGTACAAG | AAAGCTTGCT |
| 5050       | 5060       | 5070        | 5080       | 5090       | 5100       |
| *          | *          | *           | *          | *          | *          |
| CCGACGCCGT | TAACCCTCCG | ACCATCACCG  | AAGCTAATAC | TCAGGTTAGC | TTTTAATTAA |
| 5110       | 5120       | 5130        | 5140       | 5150       | 5160       |
| *          | *          | *           | *          | *          | *          |
| TACACCTAGC | TAGCTAGTTC | GTTAATTACT  | TAATTTCTTC | TTCTTTTAGT | TATCTGACCT |
| 5170       | 5180       | 5190        | 5200       | 5210       | 5220       |
| *          | *          | *           | *          | *          | *          |
| TTTTTTCACC | TCTTGTAACA | ATGATGGGAT  | CGAAATTGAT | GAAGTACTAT | CAGCAAGAGG |
| 5230       | 5240       | 5250        | 5260       | 5270       | 5280       |
| *          | *          | *           | *          | *          | *          |
| CGTCTAAACT | CCGGAGACAG | ATTCTGGGACA | TTTCTAATTT | GAACAGACAC | ATTCTTGGTG |
| 5290       | 5300       | 5310        | 5320       | 5330       | 5340       |
| *          | *          | *           | *          | *          | *          |
| AATCTCTTGG | TTCTTGAAC  | TTTAAGGAAC  | TCAAGAACCT | TGAAAGTAGG | CTTGAGAAAG |
| 5350       | 5360       | 5370        | 5380       | 5390       | 5400       |
| *          | *          | *           | *          | *          | *          |
| GAATCAGTCG | TGTCCGATCC | AAGAAGGTAC  | ATCACTAACT | CTCCATCAAT | CTCCTTATCA |
| 5410       | 5420       | 5430        | 5440       | 5450       | 5460       |
| *          | *          | *           | *          | *          | *          |
| TTGAATATAT | ATCCATCTGA | TTCTTGCCCG  | TTATATTTGG | TTTTTCTCTC | CAGCAGGAGA |
| 5470       | 5480       | 5490        | 5500       | 5510       | 5520       |
| *          | *          | *           | *          | *          | *          |
| TGTTAGTTGC | AGAGATTGAA | TACATGCAAA  | AAAGGGTAAA | AGTAAAACCT | ATCTTCCTTC |
| 5530       | 5540       | 5550        | 5560       | 5570       | 5580       |
| *          | *          | *           | *          | *          | *          |

exon 3

exon 4

exon 5

FIG. 8F

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ACAATGAACT ACCCCTACTT TATTAGCAAC TTCTCTTTCT GATGATCATC TTTTATTATT  
5590 5600 5610 5620 5630 5640  
\* \* \* \* \*  
TCTGTGTGCG CTGCAATTGT AGGAAATCGA GCTGCAAAAC GATAACATGT ATCTCCGCTC  
5650 5660 5670 5680 5690 5700 exon 6  
\* \* \* \* \*  
CAAGGTTTTA TACATAACTC TTTTGGGCAT TTTTGATCAT CATTTTTTTC CGGTAGACAA  
5710 5720 5730 5740 5750 5760  
\* \* \* \* \*  
TCTCTTGATG TGCAAATTCT AAATATCTCT GCAGATTACT GAAAGAACAG GTCTACAGCA  
5770 5780 5790 5800 5810 5820  
\* \* \* \* \*  
ACAAGAATCG AGTGTGATAC ATCAAGGGAC AGTTTACGAG TCGGGTGTTA CTTCTTCTCA exon 7  
5830 5840 5850 5860 5870 5880  
\* \* \* \* \*  
CCAGTCGGGG CAGTATAACC GGAATTATAT TCGGGTTAAC CTTCTTGAAC CGAATCAGAA  
5890 5900 5910 5920 5930 5940  
\* \* \* \* \*  
TTCTTCCAAC CAAGACCAAC CACCTCTGCA ACTTGTGGA TTCAGTCTAA CATAAGCTTC  
5950 5960 5970 5980 5990 6000  
\* \* \* \* \*  
TTCTCTCAGC CTGAGATCGA TCTATAGTGT CACCTAAATG CGGCCGCGTC CCTCAACATC  
6010 6020 6030 6040 6050 6060  
\* \* \* \* \*  
TAGTCGCAAG CTGAGGGGAA CCACTAGTGT CATACGAACC TCCAAGAGAC GGTTACACAA  
6070 6080 6090 6100 6110 6120  
\* \* \* \* \*  
ACGGGTACAT TGTIGATGTC ATGTATGACA ATCGCCCAAG TAAGTATCCA GCTGTGTTCA  
6130  
\*  
GAACGTACGT CCGAATTC

FIG. 8G

## INTERNATIONAL SEARCH REPORT

national Application No

PCT/US 98/13208

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/29 C12N15/82 A01H5/00 A01H5/10

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                               | Relevant to claim No |
|----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| X        | YANOFSKY M. ET AL.: "The protein encoded by the Arabidopsis homeotic gene agamous resembles transcription factors"<br>NATURE,<br>vol. 346, 5 July 1990, pages 35-39,<br>XP002082122<br>cited in the application<br>see the whole document<br>--- | 17,21                |
| X        | WO 94 23043 A (COUPE SIMON ALLAN ; ROBERTS JEREMY ALAN (GB); ISAAC PETER GEOFFREY)<br>13 October 1994<br>* see the whole document, esp. example 5 *<br>---                                                                                       | 24,28,29             |
| X        | WO 97 13865 A (PLANT GENETIC SYSTEMS NV ; ULVSKOV PETER (DK); CHILD ROBIN (GB); ON)<br>17 April 1997<br>see the whole document<br>---                                                                                                            | 24,28,29             |
| -/--     |                                                                                                                                                                                                                                                  |                      |

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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- "A" document defining the general state of the art which is not considered to be of particular relevance
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- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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- "&" document member of the same patent family

Date of the actual completion of the international search

26 October 1998

Date of mailing of the international search report

10/11/1998

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## INTERNATIONAL SEARCH REPORT

In tional Application No

PCT/US 98/13208

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                            | Relevant to claim No. |
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Information on patent family members

International Application No

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